EVALUATION OF GROWTH IN *PENAEUS MONODON* FABRICIUS BY INCORPORATION OF SELECTED NONHORMONAL GROWTH PROMOTERS IN THE DIET

BY
M.P.VINOD

THESIS

submitted in partial fulfilment of the requirement for the degree

MASTER OF FISHERIES SCIENCE

FACULTY OF FISHERIES

KERALA AGRICULTURAL UNIVERSITY
DEPARTMENT OF AQUACULTURE
COLLEGE OF FISHERIES
PANANGAD- KOCHI

1997

70

MY PARENTS

DECLARATION

I hereby declare that this thesis entitled **"EVALUATION OF GROWTH IN *PENAEUS MONODON* FABRICIUS BY INCORPORATION OF SELECTED NONHORMONAL GROWTH PROMOTERS IN THE DIET "** is a bonafide record of research work done by me during the course of research and that the thesis has not previously formed the basis for the award to me of any degree, diploma, associateship, fellowship or other similar title of any other University or Society.

Panangad, **M.P. VINOD**

08-01-1997.

CERTIFICATE

Certified that this thesis entitled " **EVALUATION OF GROWTH IN *PENAEUS MONODON* FABRICIUS BY INCORPORATION OF SELECTED NONHORMONAL GROWTH PROMETERS IN THE DIET**" is a record of research work done independently by Sri. M. P. Vinod under my guidance and supervision and that it has not previously formed the basis for the award of any degree, fellowship or associateship to him.

Panangad,

08-01-1997

Dr.SUSHEELA JOSE

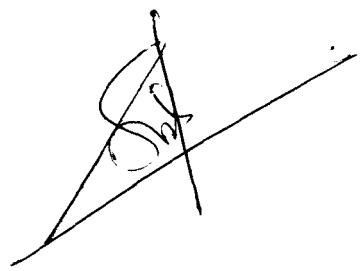
(Chair person, Advisory Board)
Assoc. Professor (Aquaculture)
College of Fisheries,
Panangad,
Kochi.

ADVISORY COMMITTEE

NAME AND DESIGNATION **SIGNATURE**

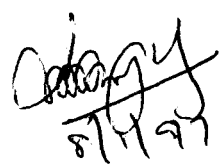
CHAIRPERSON

Dr. Susheela Jose,
Associate Professor,
Department of Aqua Culture,
College of Fisheries,
Panangad, Kochi.

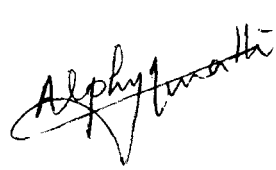


MEMBERS.

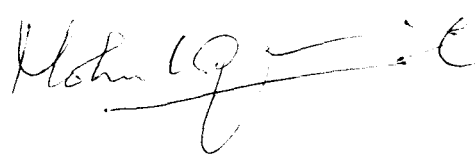
Dr. D. M. THAMPI,
Professor and Head,
Department of Aquaculture
College of Fisheries,
Panangad.



Smt. Alphi Korath
Assistant Professor(statistics)
Department of Management Studies,
College of Fisheries,
Panangad.



Dr. C. Mohanakumaran Nair
Assistant Professor,
Department of Aquaculture,
College of Fisheries,
Panangad.



ADVISORY COMMITTEE/EXAMINATION COMMITTEE

Approved the thesis

NAME AND DESIGNATION — SIGNATURE

CHAIRPERSON

Dr. Susheela Jose,
Associate Professor,
Department of Aquaculture,
College of Fisheries,
Panangad, Kochi.

7/4/97

MEMBERS

Dr. D.M.Thampi,
Professor and Head,
Department of Aquaculture,
College of Fisheries,
Panangad.

7/4/97

Smt. Alphi Korath,
Assistant Professor (Statistics),
Department of Management Studies,
College of Fisheries,
Panangad.

7/4/97

Dr. C.Mohanakumaran Nair,
~~Assistant~~ Professor,
Department of Aquaculture,
College of Fisheries,
Panangad.

EXTERNAL EXAMINER

7/4/97

Dr. A. LAXMINARAYANA
Senior Scientist,
C.M.F.R.I,
Tatapuram, Cochin-14

ACKNOWLEDGEMENT

This thesis is the outcome of the most valuable guidance which I received from **Dr. Susheela Jose**, Associate Professor (Aquaculture), College of Fisheries, Panangad. I remain grateful to her for the timely advice and constant encouragement throughout the course of my study. Her patient hearing and assistance helped me a lot in preparation of the manuscript.

I am grateful to **Dr. D.M. Thampi**, Dean i/c, Professor and Head, Department of Aquaculture for providing necessary facilities for the successful conduct of research work.

I am grateful to **Dr. C. Mohanakumaran Nair,** Associate Professor, Department of Aquaculture for the valuable help and support during the course of study. I am also thankful to **Smt. Alphi Korath**, Assistant Professor, Management Studies for the critical suggestions which helped me a lot in preparing the thesis in the present form.

I owe a great deal to **Dr. K.G. Ramachandran Nair**, Principal Scientist Central Institute of Fisheries Technology, Cochin, who spared his valuable time and allowed me to partake his profound knowledge. I am indebted to him for the timely supply of chemicals and for arranging necessary facilities without which I would not have been able to complete my research work.

I wish to extend my deep sense of gratitude to **Dr. M.J. Sebastian**, our former Dean, for the valuable suggestions given during his tenure in my advisory board.

My special thanks also due to **Dr. M.M. Jose**, Associate Professor, and **Dr. K.S. Purushan,** Associate Professor, Fisheries Station, Puduveypu, for the kind assistance rendered during difficult times.

I also thank **Dr. Jayasree Vadhyar**, Associate Professor (Aquaculture), **Dr. Thresiamma James**, Assistant Professor (Algology) and **Dr. S. Shyama**, Assistant Professor (Aquaculture) for the valuable guidance offered during the course of this study.

The assistance rendered by the library staff of College of Fisheries, Central Marine Fisheries Research Institute and Central Institute of Fisheries Technology are gratefully acknowledged.

I would like to acknowledge the whole hearted support from my classmates and friends, especially **Mr. A. Neelakanteswar, Mr. Biju. A.R, Mr. Anil .T.** and **Mr. Madhu .V.R.,** which helped me to complete the work in time.

I wish to place on record my sincere thanks to **M/s Aquasoftware**, Cherai for their nice piece of work in printing of the thesis.

I am also grateful to **ICAR** for the Junior Research Fellowship offered to me during the course period.

M.P. Vinod.

CONTENTS

LIST OF TABLES

LIST OF FIGURES

INTRODUCTION

I. INTRODUCTION

The world shrimp production has reached 712000 MT in 1995, 57% of which is contributed by the tiger shrimp *Penaeus monodon* Fabricius (Rosenberry, 1995). A major share of this production is from Asia, especially from the south east Asian countries, where it is cultured either in extensive or intensive systems. It is the best suited candidate species for large scale farming in estuarine and brackish water ecosystems due to tolerance to wide range of environmental conditions, fast growth rate, efficient feed conversion and high market demand.

The fast pace in the development of shrimp culture can be mainly attributed to the advent of artificial feeds which has become the key to its success. Feed is normally the largest single item in the running expenditure of the shrimp farm and accounts for 40- 60% of its total operational cost. Hence the development of an efficient low cost ration which gives maximum production within a minimum period is of paramount importance for commercially viable shrimp farming. This has led to intensive works in the field of nutritional research all over the world to explore the possibility of suitable combination of known nutrients and additives. Growth promoters are one among the new class of additives which can play a vital role in improving the performance and efficiency of the commercial feeds.

Growth promoters are non-nutritive materials which at very low levels of incorporation in the feed increases the feed utilisation (Viola and Arieli, 1987). The addition of these substances were found to improve the performance and feed efficiency by 10- 20%. To be effective as a commercial growth promoter it must be

REVIEW OF LITERATURE

cheap, capable of being delivered in the feed and should have nil residues in the flesh when sold.

Growth promoters have been used quite extensively in the meat producing industry over a long period. In the field of aqua culture their application is rather recent and limited. They are incorporated in the feed for salmonids, channel cat fish and tilapia. The benefit of using growth promoting substance is that they help to produce a higher growth within a short duration while improving feed utilisation.

The important growth promoters currently used in aquaculture feeds are antibiotics, hormones and enzymes. However the growing concern over evolution of resistant strains of bacteria due to continued application of antibiotics and the fear of residues of steroid hormones in the tissue together with the limited use of enzymes necessitated the identification of new growth promoters which are safe to be used. This has led to the utilisation of crustacean shells or its derivatives as substitutes for hormones and antibiotics in aquaculture feeds.

The important shell derivatives are chitin, chitosan and glucosamine. Chitin is a linear homopolysaccharide composed of N- acetyl-D- glucosamine residues in β-linkage. After cellulose, chitin is the most abundant natural polymer. The major sources of chitin in the sea food industry are the exoskeletons of shrimps, crabs and lobsters and also squid pens and cuttle fish bones. Chitin also acts as a precursor of chitosan, its most familiar derivative.

Chitin and its derivatives have wide application in various fields such as waste water treatment, agriculture, food industry, medical care, cosmetics, paper and

textile industry, Chromatography and photography. They are also used in the field of animal husbandry as growth promoter in poultry feeds.

The growth promoting property of these products in fin fishes and shell fishes is well documented. Several Japanese scientists have pointed out their significance in the diet for the kuruma shrimp, *Penaeus japonicus*. So far the information regarding the applied benefits of chitin or its derivatives for *P. monodon* is scarce and hence such a knowledge will be of immense value in the formulation of efficient artificial feed for the tiger shrimp, which would not only cut down the expenditure on feeds but may help to achieve a higher growth within the normal rearing period.

The objective of the present study is to evaluate the comparative efficiency of chitin and its derivatives, chitosan and glucosamine, in enhancing the growth in *P. monodon*. The study also aims in identifying the optimum level of each growth promoter for maximum growth.

II. REVIEW OF LITERATURE

2.1. Dietary intake of chitin

Studies on the food and feeding habits of prawns showed that the diet of penaeid shrimps contain fairly high amount of crustacean matter. A number of workers including Gopalakrishnan (1952); Dall (1968); George (1972 b); Marte (1980) and Das *et al.* (1982) who have analysed the gut contents of penaeid prawns found remains of small crustaceans and shells of several species of mollusc contributing the bulk of the food item. Boddeke (1983) reported that gut contents of *P. duorarum* contained up to 94% crustacean prey fragments. The exoskeletons of crustaceans are made up of chitin and hence it comprises a significant portion of the natural diet of shrimps. Frakenburg and Smith (1967) have opined that shrimps exhibit coprophagy. They observed that crustacean faeces contain chitin which is derived from the peritrophic membrane and also due to poor digestion.

Chitin is also commonly found in prepared shrimp diets as a constituent of crustacean meals which typically contain 10-20 % chitin (Lovell *et al.*, 1968). It is also known that shrimps consume cast exuviae following ecdysis (Hanson and Goodwin, 1977; Fox, 1993). All the above observations suggest that shrimps may be able to utilise dietary chitin which necessitates the presence of chitinolytic enzymes in the digestive tract of marine animals consuming chitinous materials.

2.2. Role of chitinolytic enzymes in chitin degradation

The biological decomposition of chitin is carried out by a series of enzymes. These enzymes are referred to as chitinases (EC 3.2.1.14) and chitobiases (EC 3.2.1.30). Chitinases also classified as an endo - β -N- acetyl glucosaminidase, splits chitin into N-acetyl-D-glucosamine dimers and trimers. These may be further broken down by exo-N-acetyl-β-D-glucosaminidase (chitobiase, NAG ase) (Jeuniaux, 1966; Fange *et al.*, 1979). The bacteriolytic enzyme lysozyme (EC 3.2.1.17) also has a chitinolytic action although weaker than that of chitinases (Rupley, 1964; Fange *et al.*, 1979).

Chitinases occur in the digestive system of certain invertebrates (Tracey, 1954; Elyakowa, 1972), while chitinolytic activities in the digestive tract of fin fishes have been reported by many authors (Seki and Taga, 1963; Okutani *et al.*, 1964; Sera and Okutani, 1968; Peres, 1973; Micha *et al.*, 1973; Goodrich and Morita, 1977a; Fange *et al.*, 1979; Danulat and Kausch, 1984; Danulat, 1986; Kono *et al.*, 1987; Clark *et al.*, 1988).

Measurable levels of chitin degrading enzymes have been reported in the alimentary tract of various crustacea(Lee and Lawrence, 1982; Chandramohan and Thomas, 1984; Kono *et al.*, 1990; Lynn *et al.*, 1990; Koga *et al.*, 1990; Spindler-Barth *et al.*, 1990; Fox, 1993; Clark *et al.*, 1993). Chitinolytic enzymes are not only involved in resorption of parts of old cuticle during the moulting cycle, but they also function as digestive enzymes (Kono *et al.*, 1987). The age or the life cycle stage of shrimp is one of the important factor influencing the digestive enzyme activities. Lee (1988) has observed that the specific activity of chitinase, which is low during the

nauplius stage of *Penaeus monodon* increased to the highest value during the mysis stage and consequently reduced, after becoming post larvae. The chitinase and chitobiase activity were quantified in *Palaemon serratus* integument and midgut gland during the moulting cycle (Spindler-Barth *et al.*, 1990). High enzymatic activity in the digestive tract at the time of exuviation was interpreted as an adaptation to the continuously high demand for food utilisation.

The presence of chitinase in fin fishes has been attributed either to gut chitinoclastic bacteria (Okutani, 1966; Goodrich and Morita, 1977b) or to the fish tissue (Jeuniaux, 1966; Lindsay *et al.*, 1984; Danulat, 1986). A similar conclusion has been made in the case of Penaeid shrimps also. They do possess a gut microflora capable of producing chitinases (Hood and Mayers, 1974; New, 1976; Dempsey and Kitting, 1987). Literature values for total number of bacteria found in the digestive tract of shrimps vary from $7.5\times10^{4}g^{-1}$ in *Penaeus indicus* (Chandramohan and Thomas, 1984) to 2.9×10^{7} g^{-1} in *P. setiferus* (Hood and Mayers, 1973). Of these 67% of bacteria in *P. indicus* showed chitinoclastic activity while 85% of micro-organisms in the gut of *P.setiferus* produce chitinase. The low percentage of occurrence of chitinoclasts observed in prawns in comparison to fin fishes may possibly be attributed to the composition of food, ie., the food may contain a low level of chitinous material (Chandramohan and Thomas, 1984). They regarded the members of the genera *Vibrio* and *Aeromonas* to be the major chitinoclasts.

In insect research chitinase activity has been confirmed not only to bacterial origin but also from endogenous secretions (Febvay *et al.*, 1984). An endogenous

origin for chitinase in penaeid shrimp *P. japonicus* has been reported by Kono *et al.* (1990) and Koga *et al.*, 1990).

Chitosan has also been identified as a substrate for chitinases by Jeuniaux (1966). Chitosanases are a new class of enzymes found to be essential for its degradation. Fenton *et al.* (1978) have studied the microbial degradation of chitosan and found that a wide variety of micro-organisms from soil and water were capable of degrading it. The role of chitosanolytic micro-organisms as a source of chitosanase was studied by Berkley (1978). Kono *et al.* (1987), however, failed to get any chitosanase activity from the digestive tract of fin fishes.

2.3. Utilisation of prawn shell in shrimp feeds

The use of prawn shell and its derivatives for preparation of feeds for prawn culture appears to be very promising. Balazs (1973) found that diet containing shrimp meal at 18.5-45 % improved the growth rate of *Penaeus azticus*, *Penaeus japonicus* and *Macrobrachium rosenbergii*. Forster and Beard (1973) opined that shrimp meal is nutritionally superior to fish meal in the diet of *Palaemon serratus* due to its amino acid profile. Higher growth and survival values were obtained in *P. duorarum* fed on diets containing shrimp meal (Sick and Andrews, 1973). But Balazs *et al.* (1974b) observed that shrimp meal alone is not as good as a combination of tuna meal, soy meal, and shrimp meal for *Macrobrachium rosenbergii*. Higher growth rate was obtained for *Penaeus indicus* by Colvin (1976a), when whole prawn was incorporated as a protein source. By using pelleted feeds consisting of prawn shell, fish meal, and high marsh grass. Venkataramaiah *et al.* (1978) obtained a growth of 31.4 % in length and 137.9 % in weight in *P. aztecus*. According to them the

presence of chitin or its break down product glucosamine in the diet might have improved the growth rate. Pascual and Destajo (1978) identified shrimp head meal as one of the important protein source in the diet for *P. monodon*.

Ahamad Ali (1982) reported relative efficiencies of feeds compounded separately with prawn waste and mantis shrimp protein for rearing *P. indicus*. Ahamad Ali and Mohamed (1985) using a diet containing 25 % prawn waste and 35 % mantis shrimp obtained 75.7% length increment and 400 % increase in weight in *P. indicus* juveniles. They observed a relative preference for flavour of prawn waste which may be possibly enhancing the palatability of the feeds. Utilisation of prawn waste in feeds for culturing prawns were also reported by Ahamad Ali and Sivadas (1983) and Mohamed *et al.* (1983). Cruz-Suarez *et al.* (1993) also obtained a higher growth rate and survival in *P. vannamei* when fed with a diet containing 18 % shrimp by-product meal. During a 42 day feeding trial in *P. monodon*, Agung *et al.* (1995) established that diet containing scallop waste and shrimp head meal as major protein sources to be superior in quality.

However the negative effect of chitin based feeds have been reported by a few workers. Simpson *et al.* (1981) observed that chitin and calcium carbonate present in exoskeleton of prawns may have deleterious effects on the overall nutritional quality of diet affecting growth and survival. Raman *et al.* (1982) reported poor performance of *P. indicus* when fed on prawn factory waste, compared to fish meal. AQUACOP *et al.* (1989) also reported lower digestibility of shrimp meal compared to fish meal, squid meal and soybean meal for *P. monodon* while Josekutty and Susheela (1992)

reported clam meal to be superior than prawn shell waste and fish meal based diet in *P. monodon*.

The overall picture shows that chitin, which comprise 10-20 % of crustacean meals, plays a significant role in the nutritional status of the animal. The presence of chitinolytic enzymes reported by several workers points out that there can be sufficient chitin degradation in the digestive tracts of shrimps and prawns.

2.4. Utilisation of chitin and chitosan

It was Oke *et al.* (1978) who first used crustacean shell or its derivatives for growth enhancement in animals. He observed that prawn shell incorporation at a rate of 10 % of the feed increased growth of rats. However he noted a significant reduction in the actual effect of chitin as a growth promoter, due to the presence of calcium in the prawn shell. Jeuniaux and Cornelius (1978) also observed chitin digestion by young chicken (15 days old) when purified chitin was added to the diet. They also reported that 19-58 % of purified ground shrimp chitin was digested by mouse (*Mus musculus*), chicken (*Gallus gallus*) and Japanese nightingale (*Liothrix lutea*). Anon (1991) identified chitin and chitosan as good growth promoters for poultry. Nair (1993) obtained a higher growth rate and food conversion when chitin was incorporated at a rate of 0.5 % in the diet of poultry.

Chitin was also incorporated in the diets of fishes and shrimps by several workers. Lindsay *et al.* (1984) failed to get any growth improvement in rainbow trout *Salmo gairdneri* at any level (4-25 %) of chitin. They found that enzyme activities were not enhanced when live chitinolytic bacteria (*Vibrio alginolyticus*) were incorporated into the diet with 10 % chitin. Mathew *et al.* (1987) obtained

higher growth rate in *Cyprinus carpio* when prawn shell powder was included in the diet. Kono *et al.* (1987) obtained a higher growth rate in red sea bream, Japanese eel and yellow tail, by incorporating chitin in the diet at a rate of 10 %. But a lower level of 5% or a high level of 20 % chitin only had slight growth improvement than the control group. However addition of chitosan only retarded the growth indicating that inclusion of chitosan might have inhibited the process involved with digestion, absorption and assimilation of the basal diet.

Kanazava *et al.* (1970) found that addition of chitin (4%) and glucosamine (1.5%) enhanced growth and survival of *Penaeus japonicus*. Patents have been granted for use of chitin containing substances such as crustacean wastes in shrimp diets (Campbell 1973a and b). Nair (1981) obtained a higher growth rate in *P. indicus* which were fed on partially demineralised prawn shell. Vaitheswaran and Ali (1986) also obtained a significant weight gain and food conversion in *P. indicus* with 0.8 % chitin in the basal diet.

Akiyama *et al.* (1989) reported a reduction in the apparent protein digestibility (APD) of feed containing chitin and recommended a lower level of inclusion of chitin or prawn shell in the diets for *P. vannamei*. Clark *et al.* (1993) who investigated apparent digestibility of chitin in *P. vannamei*, *P.setiferus* and *P. duorarum* at 1, 2 and 4 % levels obtained values of 36 %, 33% and 52% respectively. Although statistically not significant all the three species showed lower chitin digestibility at 4 % levels compared to 1% and 2% levels. This observation may reflect a limit to the enzyme hydrolysis capabilities of penaeid shrimps. The data indicate that chitin is digested by penaeid shrimps though the extent of digestion

varies among species. In 1993, Fox has reported that when *P. monodon* juveniles were fed on diets containing chitin between 0 and 16 %, increasing levels of dietary chitin did not significantly affect the individual weight gain, specific growth rate and feed conversion ratio. He concluded that the level of growth enhancement due to dietary chitin in *P. monodon* is probably a result of low digestibility rather than an inability to absorb or metabolise glucosamine. The result also indicate that the synthesis of endogenous chitinase in the digestive gland of shrimp occurs at a slow rate and that juvenile shrimps are able to digest only small amounts of dietary chitin in the absence of bacterially produced chitinase. Ahamad Ali (1995), however regarded chitin as a good growth promoter and recommended a dietary inclusion of 0.8-1.5% for the tiger shrimp *P. monodon*.

2.5. Utilisation of glucosamine

N-acetyl glucosamine has been reported to function as a growth promoter when added to baby foods (Kent and Whitehouse, 1955; Gyorgy et al., 1955). Kanazava *et al.* (1970) found that addition of glucosamine (1.5 %) and chitin (4%) in the diet of *P. japonicus* significantly improved the growth rate and survival. Kitabayashi *et al.* (1971) also obtained an increased growth rate in *P. japonicus* when fed with glucosamine. Maximum growth rate was obtained at 0.53% level. He opined that excess of glucosamine check the growth of prawns. An attempt to replace glucosamine with squid pen powder or crude chitin powder or glucose was unsuccessful. However, Deshimaru and Kuroki (1974) did not find any growth promoting activity for glucosamine. They concluded that glucosamine by no means is an essential material to be compounded in the diet. Contrary to this finding

Vaitheswaran and Ali (1986) obtained significant growth difference in *P. indicus* when glucosamine was incorporated in the diet at 0.8% level. Ahamed Ali (1995) also regarded glucosamine as good growth promoter and recommended a dietary inclusion of 0.8% for the tiger shrimp *P. monodon*.

2.6. Other growth promoters

2.6.1. Enzymes.

Jancarik (1964) observed that exogenous proteolytic enzymes originating from invertebrate food organisms play a role in fish digestion in addition to the activation of fish's own enzymes. First the activity of pepsin, and trypsin like enzymes of potential fish food organisms was determined by Dabrowski and Glogowski (1977a). Later they investigated the role that the enzymes play in fish digestion. Dietary enzyme supplements seems to be especially important in juveniles which may lack some important enzymes.

Boettcher (1985) reported gain in weight in fishes due to enzyme supplementation. Recently Bogut *et al.* (1995) who used polyzymes- a stabilised mixture of amylase, protease, β-gluconase, β-glucosidase and cellulase- obtained significant weight gain in *Cyprinus carpio* at 1.5 Kg / tonne feed level.

Attempts to link the digestive enzyme activities and crustacean growth have not been fully successful. In some studies a positive correlation between digestive enzyme activities and shrimp growth were established (Van Wormhoudt *et al.*, 1980; Maugle *et al.*, 1983a; Chuang *et al.*, 1985), while certain other studies showed that

shrimp with superior growth need not necessarily possess higher protease, lipase, or amylase activities (Lee *et al.*, 1984; Chen and Lin, 1990).

Maugle *et al.* (1983a) obtained better growth rate in *P. japonicus* when fed with 60 IU α-amylase per gram dry diet than diet with no amylase or a control diet of live short necked clam Maugle *et al.* (1983b) with microencapsulated amylase and bovine trypsin obtained superior growth rate in *P. japonicus* juveniles. *P. monodon* enzyme acetone powder supplemented to the test diet yielded significantly higher growth than the control diet and diet containing *Artemia* enzyme acetone powder (Chen and Lin, 1990). However higher inclusion of *P. monodon* enzyme did not result in better growth.

Papain a common proteolytic enzyme extracted from the latex of *Carica papaya* has been found to be a growth enhancer when added to the diet of prawns at 0.1-0.2% (Paul Raj, 1993). Dy Penafloria (1995) who used papaya leaf meal in the diet for *P. monodon* obtained higher growth rate at 10% level. Ahamad Ali (1995) recommended the use of papain in *P. monodon* diet at 0.1-0.2% level. In 1996, Boby and Susheela have reported papain to be a growth enhancer for *Macrobrachium rosenbergii.*

Bromolein, an enzyme extracted from pineapple was reported to improve the growth of prawns at 0.1-0.2% level (Paul Raj, 1993). Ahamad Ali (1995) has also recommended the same level of incorporation in the diet of *P. monodon.*

2.6.2. Antibiotics.

Antibiotics possess properties that cause various reactions which bring about specific chemical and physiological changes in the animal (Hall, 1962).Several mechanisms of growth promotion by antibiotics have been propounded so far, but the widely accepted view is that the effect of antibiotics upon growth is initiated by modification of the enteric flora (Luckey, 1959). There is no dispute, however, over the fact that antibiotic feeding results in thinning of the intestinal wall (Dubos *et al.* 1967; Jukes, 1973). Protein sparing action of the antibiotic has been postulated by Visek (1978) and Ahamad and Matty (1989). It can be concluded that the mode of antibiotic action as a growth stimulant is multifaceted and that alteration of enteric flora is not a complete explanation.

The EEC (1985) has classified the antibiotics into two groups, therapeutic and feed antibiotics. Feed antibiotics are permitted for inclusion at low levels in commercial diets of animals over long period.

Wagner (1954) was the first to study the effect of antibiotics on the growth of fishes. Since then a number of antibiotics have been tried successfully as growth promoters in fin fishes by several workers (Rijikers *et al.*, 1980; Viola and Arieli, 1987; Ahamad and Matty, 1989; Cravedi *et al.*, 1991; Keshavanath *et al.*, 1991; Anicic *et al.*, 1995; Zoccarato *et al.*, 1995).

Antibiotics have been tried as a growth promoter in crustaceans too. Ciapara *et al.* (1989) obtained higher growth rate in *Penaeus monodon* when fed with 250 ppm of

oxytetracycline. Stuck *et al.* (1992) also used it at 25-50 ppm level and obtained significant weight gain in *P. vannamei* over the control group. He, however, failed to get any growth enhancement with antibiotic penicillin / streptomycin at any level of incorporation into the diet. In *Macrobrachium rosenbergii*, 100 ppm of oxytetracycline has been found to promote growth (Boby and Susheela, 1996). But Corliss *et al.* (1977) failed to get any significant growth enhancement when oxytetracycline was incorporated in the diet for *P. aztecus*. Later Vaitheswaran and Ali (1986) also reported a similar result due to its administration in *P. indicus*.

Trevino-Carrillo *et al.* (1993) obtained significant growth in *P. vannamei* with virginiamycin at 80, 100 and 200 ppm levels compared to those fed on 50ppm level and control diet. The tissue samples did not show any residues of virginiamycin even at the highest level of 200 ppm. However, Utama and Musa (1991) did not find any growth stimulation at a level of 80 ppm of Virginiamycin in the diet for *P. monodon*.

The variation in the efficiency of antibiotics even in the same species is explained by Visek (1978) who pointed out that the response in weight gain due to antibiotic supplementation in animals diets may be varied depending on the age of the animal, the antibiotic used and its dosage, type of feed used and the nutrition of the recipient animal.

Anyway use of antibiotics freely in commercial feeds is not permitted because administration of antibiotic to farm animals could pose hazard to human health due to potential development of resistant strains of enteric bacteria (Varghese and Gireesha, 1994; Ahamad Ali, 1995; Hatha and Lakshmana Perumal samy, 1995).

2.6.3. Hormones.

Natural hormones are specific chemical substances produced by living cells. They have a property of being effective even when present in extremely minute amounts. Steroid hormones such as androgens, estrogens and progestrones and nonsteroid hormones such as thyroxine, growth hormone etc. are used as growth promoters in animal feeds (Donaldson *et al.*, 1979). Synthetic estrogens such as stilbistrol, diethyl stilbistrol etc. are widely used for stimulating growth in meat raising animals. Growth promoting efficiency of hormones in fishes has been reviewed by Donaldson *et al.* (1979); Matty and Lone (1985) and Pelissero and Sumpter (1992).

2.6.3.1. Growth hormone.

There is evidence that growth hormone enhances fish growth by stimulating greater voluntary food intake by improving food conversion or by inducing the formation of stomatomedin which acts at the cellular level (Donaldson *et al.*, 1979). Wilson *et al.* (1988) and Heyward (1991) reported bovine and porcine growth hormones to be efficient in promoting growth rate in fin fishes.

Toullec *et al.* (1991) opined that human growth hormone like peptides seem to be present in *P. indicus* which is similar to that of vertebrate growth hormones. They observed a positive effect on size of *P. vannamei* when human growth hormone was supplemented through the diet.

2.6.3.2. Thyroid hormone.

Two forms of thyroid hormone, triiodo thyronine (T_3) and thyroxine (T_4) have been found to produce increased growth rate in fishes. Thyroid hormone treatment enhances growth by increasing appetite and / or gross food conversion efficiency (Donaldson *et al.*, 1979).

The application of thyroid hormone in fish culture as growth promoter has been reviewed by Donaldson *et al.* (1979); Higgs *et al.* (1982); Mc Bride *et al.* (1982) and Matty and Lone (1985). T_3 has been found to be a growth enhancer in *Oreochromis mossambicus* (Chaudhury *et al.*, 1989; Hoverton *et al.*, 1992), *Oncorhynchus tshawytscha* (Ashraf and Meade, 1993) while it retarded the growth in *Ictalurus punctatus* (Gahuam and Lovell, 1991) . Lam and Sharma (1985) did not find any growth enhancement in *Cyprinus carpio* with thyroxine. Negative results were obtained by Lam *et al.* (1985) for *Chanos chanos* and Gahuam and Lovell (1991) for *Ictalurus punctatus* due to thyroxine incorporation in the feed.

Thyroid hormones were tried as growth promoter in crustaceans too. Pillai *et al.* (1987) observed that thyroxine in microquantities can bring about faster growth by increasing the moulting frequency in *Penaeus monodon*. They obtained maximum growth rate at a concentration of 5µg / l of water. However, Vaitheswaran and Ali (1986) did not find any growth increment in *P. indicus* when fed with 1mg / kg thyroxine and opined that thyroxine is not a suitable growth promoter for shrimps or prawns. Similar result was obtained in *Macrobrachium rosenbergii* too with thyroxine (Boby and Susheela, 1996).

2.6.3.3. Mammalian gonadotropin.

Human chorionic gonadotropin (HCG) have been found to be an effective growth promoter in cultivable fin fishes. Shyama and Keshavanath (1990) found HCG to enhance the growth of silver carp (*Hypophthalmichthys molitrix*) and Mahseer (*Tor khudree*) at 20 ppm level. Similar results were obtained by Salini (1993) in *Labeo rohita*, Kesavanath and Matty (1994) in *Cyprinus carpio* and Jayaprakas and Sambhu (1996) in *Etroplus suratensis*. Jayaprakas and Bindu (1996) reported that administration of HCG at 10 ppm and combination of HCG and testosterone propionate at 8 ppm and 1.5 ppm respectively improved growth rate and food conversion of *Cirrhinus mrigala*.

HCG has been tested in shrimps as well. However, 20 ppm HCG has been found to inhibit the growth of *P. indicus* (Sambhu and Jayaprakas, 1994b).

2.6.3.4. Steroid hormones.

The effect of anabolic agents is generally defined as an increase in nitrogen retention. There are two possible sources of increased nitrogen: increased food intake and improved food utilization (Donaldson *et al.*, 1979). The most widely used androgenic steroids are ethyl estrenol, 17 α- methyl testosterone and testosterone. Important estrogenic steroids include estradiol and diethyl stilbistrol.

The effect of anabolic steroids on mammals have been reviewed by Kruskemper (1968) and their use in fishes have been reviewed by Donaldson *et al.* (1979); and Pelissero and Sumpter (1992). Growth promotion in fin fishes by androgenic steroids has also been reported by Nirmala and Pandian (1983); Chua and

Teng (1988); Hoverton *et al.* (1988); Gogoi and Keshavananth (1988); Hoverton *et al.* (1992); Ashraf and Meade (1993) and Satandren and Diaz (1994).

Estrogenic steroids have also been identified as effective growth promoters in fishes by Nirmala and Pandian (1983); Nanjundappa and Verghese (1988); Shyama and Keshavanath (1990); and Satoh and Nimura (1991).

Vaitheswaran and Ali (1986) reported significant weight gain in *P. indicus* by incorporation of testosterone into its diet at 25 ppm level. However 17 α-methyltestosterone failed to bring about any growth gain in crustaceans even at level as high as 125 ppm (Antiporda, 1986).Josemon (1990) also did not find any growth enhancement in *P. indicus* due to testosterone incorporation.

Ethyl estrenol has also been tried as a growth promoter in crustaceans. But Vaitheswaran and Ali (1986) did not find any growth enhancement in *P. indicus* due to its incorporation, although Sri Prakash *et al.* (1987) obtained higher growth rate in *Macrobrachium choprai* with this hormone. Similar results were obtained by Raghunathan et al (1992) in *P. indicus* at 8 ppm level.

There are strict legislation against the use of steroid hormones in animal feeds in many countries. The risks of using steroid hormones in animal feeds are the side effects in the recipient animal (Donaldson *et al.*, 1979) and potential health hazards to consumers. Residues of synthetic estrogens in carcasses are found to have carcinogenic properties in humans (Ahamad Ali, 1995).

2.6.4. Attractants.

Supplementation of attractants in the diet improves food intake and utilization. Shrimp, fish extract and mussel extract in purified diets have been found to be good attractants (Pascual, 1980). Squid meal (Cruz-Suarez *et al.*, 1992) and krill meal (Allahpichay and Shimizu 1984 a and b) have also been reported to be good attractants. Important groupsof attractants identified so far are discussed below.

2.6.4.1. Amino acids.

The effect of glutamic acid to elicite food intake in *Penaeus japonicus* was first reported by Takei and Ai (1971). Deshimaru and Yone (1978) observed glycine and taurine as good attractants for *P.japonicus* while glutamic acid does not seem to be effective. Smith *et al.* (1987) regarded taurine as a potential attractant for *Mcrobrachium rosenbergii* too. Kanazava *et al.* (1990) observed an enhanced appetite and growth in *P. japonicus* fed with diets containing 0.5% free amino acids such as D-L-alanine, L-proline, L-valine and L-phenyl alanine. Akiyama *et al.* (1991) too opined that free amino acids can act as attractants for shrimps and prawns. Shiau *et al.* (1992) while testing the suitability of taurine at four levels 1000, 2000, 3000 and 4000 ppm, observed a better growth rate upto 3000 ppm, while further addition only retarded the growth. Hartati and Briggs (1993) also found taurine to be a feed attractant for *P. monodon*.

2.6.4.2. Oligopeptides.

Akiyama *et al.* (1991) reported that small peptides can act as attractants for shrimps and prawns. Attempts to determine the enhancement of appetite and growth promoting effect of oligopeptides on the larvae and juvenile *Penaeus japonicus* were carried out by Kanazava (1995).

2.6.4.3. Betaine.

A synergistic action of betaine with other amino acids have been reported by Heinen (1980). Hence he recommended its inclusion in practical shrimp diets. Ung and Junilla (1988) who fed *Penaeus monodon* with betaine at 1% and 2% levels obtained a higher growth rate at both levels. Similar results have been obtained by Harpaz (1992) in *Macrobrachium rosenbergii*, Jasmine *et al.* (1993) in *P. indicus* and Shiau and Chou (1994) in *P. monodon*.

2.6.4.4. Dimethyl- β-propiothein (DMPT).

DMPT was found to stimulate growth and moulting in stripped prawn, *Palaemon serratus* at a concentration of 0.1 mM solution (Nakajima, 1991). Jasmine *et al.* (1993) also obtained increased growth rate in *Penaeus indicus* with its addition.

2.6.4.5. Squid protein extract.

Squid protein and squid protein extracts have been shown to improve both growth rate and feed conversion in penaeids (Deshimaru and Shigueno, 1972; Pascual, 1980; Cruz-Suarez et al., 1984; Dokken and Lawrence, 1985). Cruz-Suarez

and Guillaume (1983) opined that fresh whole squid can be separated into three fractions, a hydro alcohol soluble fraction, lipid fraction and protein fraction. Since the amino acid balance of the squid protein fraction supplemented diet was different from the basal diet; it was concluded that a growth promoting factor was present in the squid protein fraction. Cruz-Suarez *et al.* (1984) observed a higher growth and food conversion efficiency when squid protein fraction was incorporated in the diet of *P. japonicus* at a level of 1.5%. Cruz-Rique *et al.* (1987) also studied the growth response to mixed diets supplemented with protein extracted from frozen squid at 1.5, 3, 6 and 10% levels in four tropical penaeid species, viz, *P. stylirostris*, *P. vannamie*, *P. monodon* and *P. indicus*. They obtained significant growth rate even at lowest level in *P. vannamie* and *P. stylirostris*. This indicates the presence of unknown growth factors (UGF) in the squid extract.

2.6.4.6. Trimethyl ammonium hydrochloride (TMAH).

TMAH gives a distinctive faecal odour to feeds. This may act as a feed attractant for shrimps and prawns. Increased feed intake and utilization on addition of TMAH in *Macrobrachium rosenbergii* feed has been reported by Costa-Pierce and Laws (1985) and in *Penaeus monodon* by Hartati and Briggs (1993).

2.6.4.7. Adenosine monophosphate (AMP).

Feeding stimulation , increased feed intake and hence higher growth rate have been reported from the Carridean shrimp *Palaemonetes pugio* due to Adenosine-5-monophosphate (Carr and Thompson, 1983). Similar results have been obtained in

Macrobrachium rosenbergii (Herpez *et al.*, 1987) and in *Penaeus monodon* (Hartati and Briggs, 1993).

2.6.5. Others.

2.6.5.1. Olaquindox.

Olaquindox, commercially known as Bayo-n-ox, is a chemical growth promoter having chemical name 2-[N-(2-hydroxy-ethyl)-carbamyl]-3-methyl-quinoxaline-1, 4-dioxidate. This chemical possesses anti bacterial properties. Santiago (1991) observed growth promotion in Nile Tilapia *Oreochromis niloticus* fed with Bayo-n-ox at 25 ppm. Jiamin *et al.* (1989) who tried Bayo-n-ox at 10 to 100 ppm levels found that zoea, post larvae and juveniles of *Penaeus orientalis* responded well to the treatment. It increased not only the survival rate but also the growth rate. They, however, did not find any difference of effects between different concentration in tested range and hence recommended a dosage of 100-300 ppm for practical feed preparation. Akiyama *et al.* (1991) too recommended its usage in shrimp feeds at 200 ppm.

2.6.5.2. Moult hormone.

The moulting hormone ecdysone which is found in insects and crustaceans has also been isolated from plants and its supplementation in fish feeds has been found to enhance growth rate in crustaceans. Kanazava *et al.* (1972) used three different ecdysones isolated from plants- Inokosterone, Cyasterone and Ecdysterone -

all of which induced moulting but growth rate was comparatively lower than that of control groups.

2.6.5.3. Alfalfa.

The leguminous forage plant *Medicago sativa*, commonly known as alfalfa is known to be a good source of Vit.K. This plant is known to contain plant estrogens which in limited amounts have a beneficial effect on the fattening of animals, similar to that of synthetic hormones such as stilbistrol and hexestrol (Mc Donald *et al.*, 1973).

Rao *et al.* (1983) who studied the effect of alfalfa on the growth of *Penaeus indicus*, observed an increased growth rate. Vaitheswaran and Ali (1986) too reported an enhanced growth rate in *P. indicus* when fed with alfalfa extract at a rate of 2 ml / 100g diet. Ahamad Ali (1995) recommended its incorporation at 2% level in the feed for *P. monodon*.

2.6.5.4. Monensin.

Hawkridge (1978) reported that monensin treatment increased weight gain in cattle while Perry *et al.* (1976) reported an improvement in feed efficiency. This carboxylic ionophore is known to improve growth in sheep, catle, poultry and pigs and combat diseases (Anon. 1990).

Monensin has also been found to be a growth promoter in *P. vannamei*. When fed at a rate of 100 ppm (Dileo Craetano Pressman, 1987). Lower dose of 50 ppm and higher dose of 200 ppm showed a decrease in growth rate.

2.6.5.5. Probiotics.

The word probiotics is often used as an opposite of antibiotics, ie, as a promoter of life. The term probiotic implies organisms and substances which contribute to microbial balance. Public disquiet over the use of antibiotics as feed additives in live stock nutrition has encouraged commercial interest in probiotics as an alternate therapy. The most commonly used probiotic in animal nutrition are lactic acid bacteria (LAB) and some strains of Bacillus (Mohamed, 1995). They act as food and also assist digestion through the presence of enzymes (Intriago and Jones, 1993). Several modes of action of these enzymes are suggested by Van belle *et al.* (1989) and Mohamed (1995).

Use of micro-organisms in mariculture is of very recent origin (Maeda, 1988). Gatesoupe *et al.* (1989) reported a higher growth rate in Japanese flounder *Paralichthys oliva*, which are fed on rotifers enriched with acosil, a commercial preparation containing lactic acid bacteria. The brine shrimp *Artemia salina* have been grown to preadults on diets containing bacteria (Flexibactor sp.) with significant increase in length and dry weight (Intriago and Jones, 1993). Factor and Dexter (1993) demonstrated that decapod larvae could ingest particles in the size range of bacteria (1-2 μm).

A study by Gariques and Arevalo (1995) in Ecuador showed that a non-pathogenic isolate of *Vibrio alginolyticus* when added to *P. vannamei* larval culture medium helped in controlling other pathogenic Vibrios (*V. parahaemolyticus*) and also improved the survival and average wet weight. Mohamed (1996) has obtained

faster metamorphosis and high survival in *P. monodon* up to PL-1 stage, by using live heterotrophic bacteria- (*Pseudomonas* sp and *Micrococcus* sp) as 50 % replacement diet for the diatom *Chaetoceros* sp. Larvae fed with 100 % bacterial biomass did not metamorphose beyond 2-3 stage or beyond 5 days.

MATERIALS AND METHODS

III. MATERIALS AND METHODS

The aim of the study was to find out the comparative efficiency of chitin, chitosan and glucosamine as growth promoters in *Penaeus monodon* juveniles. The experiment was carried out during the period from 07-05-1996 to 15-07-1996, at College of Fisheries, Panangad.

3.1. Experimental rearing facilities

The wet laboratory of Department of Aquaculture, College of Fisheries, Panangad was used. Circular flat bottom fibre glass tanks were used for rearing the experimental animals. The specification of the experimental tanks are given below.

Capacity : 83 litres

Diameter : 55cm

Height : 35cm

Rim width : 3cm

Thickness : 4 mm

Colour : Aquamarine

The tanks were housed inside the wet laboratory which had provision for subdued light penetration. Intake water was filtered and diluted to 20 ‰ and stored in three fibre reinforced plastic (FRP) tanks, each having 2t capacity, was used to fill the experimental tanks. Gentle aeration was given throughout the experimental period via diffuser stones from a roots air blower and were maintained uniformly in all the tanks by means of control valves.

Uniform sized earthen tiles were provided as artificial substrata in each tank to reduce the cannibalism among prawns. The tiles were placed in a slanting position over a small piece of stone .

3.2. Experimental animals

P. monodon post larvae were obtained from A.N.S hatchery, Cherai and were transported to the laboratory in oxygen filled polyethylene bags under minimum stress. In the laboratory they were maintained in 1t FRP tanks containing sea water of salinity 30 ‰ and provided with gentle aeration. They were reared up to the early juvenile stage in two weeks time. During this period the salinity was gradually reduced to 20 ‰ and feeding was done with artificial diet containing clam meat.

Healthy well pigmented and active juveniles were sorted out from the nursery tanks and 10 shrimps were assigned to each experimental tank randomly. They were acclimatised to tray feeding for few days. before commencement of the experiment. The experimental shrimp were starved for a day and shrimps in each tank were weighed collectively in an electronic balance. The initial weight of the shrimp

juveniles ranged from 80.7 mg to 91.27 mg and length ranged from 2.7 cm to 3.1 cm.

3.3. Growth promoters used in the study

3.3.1. Chitin

Chitin is a linear homopolysaccharide composed of N-acetyl-D-glucosamine residues in β-linkage. It is the principal component of the hard exoskeleton of arthropods. Chitin is extracted from the exoskeleton after deproteinisation and demineralisation. For the present study it was obtained from the Biochemistry and Nutrition Division of the Central Institute of Fisheries Technology, Cochin. In the experiment, chitin was incorporated into basal feeds at 3 levels, viz., 0.25g, 0.5g and 1g per 100g diet.

3.3.2. Chitosan

Chitosan is a high molecular weight linear polymer of amino-D-glucose derived from chitin by the process of deacetylation (Madhavan and Nair, 1974). For the present study it was procured from the Biochemistry and Nutrition Division of Central Institute of Fisheries Technology, Cochin. It was incorporated into the basal feed at 3 levels, viz., 0.25g, 0.5g and 1g per 100g diet.

3.3.3. Glucosamine.

Glucosamine is obtained after hydrolysis of chitin with conc. hydrochloric acid. It is available as glucosamine hydrochloride and this too was procured from the Biochemistry and Nutrition Division of Central Institute of Fisheries Technology,

Cochin. In the present study, it was incorporated into the basal feed at 3 levels, viz., 0.25g, 0.5g and 1g per 100g diet.

3.4. Preparation of experimental diet

3.4.1. Diet formulation.

To study the effect of various growth promoters on the growth of *P. monodon* juvenile, a 40% protein diet (AQUACOP, 1977) was formulated to which the specified growth promoters were incorporated at 3 levels and fed to the shrimp. The ingredient composition of the basal diet is given in Table 1.

Clam meal and defatted soyflour were used as the major protein sources. Clam meat is a good source of protein and contain maximum number of free amino acids (Surya Narayana and Alexander, 1972). Similarly the aminoacid profile of the soybean meal is identical to that of prawns (Deshimaru and Shigueno, 1972). Clam meal also contain a higher percentage of lipid and hence was the major lipid source. Shrimps can utilise dietary carbohydrate as energy source. However polysaccharides such as starch and dextrin are better than monosaccharides (Andrews *et al.*, 1972). while formulating the experimental diet wheat flour and wheat bran were used as the principal carbohydrate sources. Polyunsaturated fattyacids of linolenic and linoleic families have been recognised as very important nutrients for the growth of crustaceans (D' Abramo and Sheen, 1993). Cod liver oil was used as the PUFA source in the present study . Supplevite-M, a vitamin mineral mixture (Sarabhai Chemicals , Bombay) was used as source of vitamins and minerals. Guar gum a galactomannan polysaccharide

extracted from cluster bean *Cyamopsis tetragonolaba* was used as the binder . It was procured from Central Institute of Fisheries Technology, Cochin.

3.4.2. Diet preparation.

A 0.5% solution of guar gum was prepared first, by dissolving 0.5g of the binder in 100ml distilled water. It was then heated to 60°C in a hot plate to allow gel formation. Dry ingredients were powdered separately and sieved through a 250μ sieve. They were accurately weighed according to the percentage composition (Table 1). All ingredients together with each of the growth promoter (Table 2) were mixed thoroughly in a mortar. To this an equal quantity of binder solution was added and mixed thoroughly to get a dough like consistency. The dough was then extruded through a hand pelletiser having a die of 3mm diameter. The extruded pellets were collected in an enamel tray and dried uniformly to get dry pellets. The pellets were then broken into small pieces packed in air tight containers and stored at 4°c in a refrigerator until use.

3.5. Proximate analysis of the diet

Proximate composition of the basal diet was analysed to evaluate the nutrient status. The result is based upon the mean of three samples and are expressed on a dry matter basis except for moisture content which was on feed basis.

Boyd's (1979) method was used to estimate the moisture content of the feed sample. The sample was heated to 105° C for 30 minutes and then dried at 65° C till a constant weight was obtained. Microkjeldahl's method (AOAC, 1975) was used

to estimate the crude protein content. The nitrogen content was multiplied by a factor of 6.25 to get the protein content. Solvent extraction using petroleum ether (B.P 40 - 60° C) in a soxhlet extraction apparatus for six hours was carried out to estimate the crude fat. Crude fibre was estimated by the method described by Pearson (1976). The ash content was estimated by burning the sample at 550° C± 10° C for six hours, in a muffle furnace. The carbohydrate content was determined by difference in dry weight (Hasting, 1976).

3.6. Experimental design and procedure

Circular flat bottom fibre glass tanks were used to rear *P. monodon* juveniles. Ten numbers of uniform sized juveniles were randomly selected and introduced to each experimental tank. Three growth promoters, each at 3 levels , viz., 0.25, 0.5 and 1g per 100g basal diet were tested with three replicates for each treatment. Thus three levels of three growth promoters in three replicates together with control group, made a total of 30 tanks for the study. Treatments were allocated to each experimental unit by random allocation method.

The shrimps were provided with the feed *ad libitum* in petridishes twice daily, once in the morning between 9.00 and 10.00 am and the other in the evening 5.00 and 6.00 p.m. Petridishes were kept at the bottom of the tank close to the substratum provided. The size of the feed was adjusted according to the size of the animal. Every day before offering the feed left over feed was collected and dried at 60° C for estimation of FCR. Petridishes were cleaned thoroughly before next

Table 1. Percentage composition of ingredients used in the basal diet

Ingredients	% weights
Soyaflour	45
Clam meal	30
Whet bran	12
Wheat flour	10
Cod liver oil	1
Mineral Mix	1
Vitamin Mix	1
	100.0

Table 2. Composition of the diet used in the experiment

	T_0	T_1	T_2	T_3	T_4	T_5	T_6	T_7	T_8	T_9
Basal diet	100	100	100	100	100	100	100	100	100	100
Chitin	-	0.25	0.50	1.0	-	-	-	-	-	-
Chitosan	-	-	-	-	0.25	0.50	1.0	-	-	-
Glucosamine	-	-	-	-	-	-	-	0.25	0.50	1.0

feeding. During morning hours bottom and sides of the tanks were scrubbed well to prevent algal growth and excreta accumulation. Dead animals found were removed and weighed. The water in the rearing tank was exchanged once in a week to maintain water quality. During the experimental period shrimps were subjected to growth assessment every fortnight. At the end of the feeding study shrimps were starved for one day and the number in each tank counted and weighed separately. The body protein content of the shrimps at the beginning of the experiment and at the end of the rearing period was also estimated for all the treatments.

3.7. Determination of body protein

Body protein was estimated using microkjeldahl's method (AOAC, 1975). Tissue was digested with concentrated sulphuric acid and digestion mixture till the solution became colourless. 5ml of this solution was used for distillation. The percentage nitrogen in the sample was converted into % crude protein by using a factor of 6.25.

3.8. Water quality measurement

Physiochemical parameters of the water in the rearing tank were measured by the following methods.

1. Temperature- with bulb thermometer having an accuracy of 0.1°C.

2. pH - Universal indicator method

3. Dissolved oxygen- Standard Winkler method.

Temperature and pH were measured daily and D.O content once in a week. The samples were randomly selected from the tanks for estimation purpose.

3.9. Evaluation criteria

The parameters like net weight gain, gain in length, specific growth rate (SGR), percentage survival, food conversion ratio (FCR), protein efficiency ratio (PER), and productive protein value (PPV) were determined, in order to study the influence of various growth promoters at different levels, on the growth of *P. monodon* juveniles.

3.9.1. Net weight gain.

It gives the increase in the weight of the shrimps during the experimental period when fed on various growth promoters. It was calculated using the formula,

Net weight gain = Final weight – Initial weight.

3.9.2. Gain in length.

This gives the increase in length of shrimps during the experimental period when fed on various growth promoters. It was calculated using the formula.

Gain in length = Final length – initial length.

3.9.3. Percentage growth.

Percentage growth of the animal was calculated by using the following formula.

$$\% \text{ Growth} = \frac{\text{Final measurement - Initial measurement}}{\text{Initial measurement}} \times 100$$

3.9.4. Specific growth rate.

In the present study growth performance was measured in terms of specific growth rate (SGR) since it is a more refined and improved growth index than absolute weight gain or percentage growth rate (Hepher, 1988).

$$SGR = \frac{\ln W2 - \ln W1}{T2-T1}$$

Where,

W1 = Weight at time T1

W2 = weight at time T2

3.9.5. Survival rate.

It is expressed in terms of percentage.

$$\text{Survival } \% = \frac{\text{Initial number - Number of dead prawns}}{\text{Initial number}} \times 100$$

3.9.6. Food conversion ratio.

This refers to the ability with which an animal can convert the feed consumed into edible and other products. FCR is the most commonly used index to measure the efficiency of different diets used in the trial.

$$FCR = \frac{\text{Feed intake in a dry matter basis}}{\text{Weight gain on wet matter basis}}$$

3.9.7. Protein efficiency ratio.

Protein efficiency ratio is defined as the weight gain per unit intake of protein.

$$PER = \frac{\text{Gain in body weight}}{\text{Protein intake}}$$

3.9.8. Productive protein value.

This gives the measurement of body protein deposition in the prawns with unit amount of protein consumed.

$$PPV(\%) = \frac{\text{Final body protein - Initial body protein}}{\text{Protein consumed}} \times 100$$

10. Statistical analysis

All indices such as growth, specific growth rate, food conversion ratio, protein efficiency ratio and productive protein value were subjected to comparison using analysis of variance (ANOVA) and pair wise comparison was performed by using least significant difference (Snedecor and Cochran, 1968).

RESULTS

IV. RESULTS

The growth promoting potential of chitin, chitosan and glucosamine was evaluated on *P. monodon* juveniles at different levels. The details of the observations made during the period of study are presented below. The test diets with chitin contents at 0.25, 0.5 and 1g per 100 g diet are denoted as T_1, T_2 and T_3 while diets with similar levels of chitosan are denoted as T_4, T_5 and T_6 and same levels of glucosamine are denoted as T_7, T_8 and T_9 respectively. The control group was designated as T_0.

4.1. Proximate composition of the formulated feed

The analysis of proximate composition of the basal feed had shown that it contains 6.61 % moisture, 40.29 % crude protein, 5.66 % crude fat, 12.9 % crude fibre, 11.62 % ash and 22.92 % carbohydrate as nitrogen free extract.

4.2. Efficiency of various growth promoters

4.2.1. Growth.

The data regarding weight gain of shrimps fed on feeds containing the three growth promoters at different levels are given in Table 3.

The average live weight gain of shrimps fed on different levels of chitin were 800.67 mg, 774.29 mg and 769.74 mg respectively for T_1, T_2 and T_3. However none exhibited significant difference from the control group (783.71 mg); the daily weight enhancement being 11.44 mg, 11.06 mg, 11.00 mg and 11.20 mg respectively for T_1, T_2, T_3 and T_0. The average percentage weight increase of the juveniles from their

initial size were 931.27, 918.82, 907.47 and 927.48 % respectively for treatments T_1, T_2, T_3 and T_0. The graphical representation of growth observed in the experiment are given in Fig. 1 and growth curve based on biweekly growth are shown in Fig. 2.

Shrimps fed on all levels of chitosan have shown better growth than the control group. The average live weight gain observed were 936.23 mg, 1111.82 mg and 860.03 mg, where as the average percentage weight increase were 1058.47, 1246.03 and 996.6 % for treatments T_4, T_5 and T_6. The daily weight increments in these treatments were found to be 13.38 mg, 15.88 mg and 12.29 mg respectively. It is graphically represented in Fig. 3, while growth curve based on biweekly weight gain is shown in Fig. 4.

The average live weight gains of *P. monodon* juveniles fed on three levels of glucosamine, viz, 0.25, 0.5 and 1g / 100g diet designated as T_7, T_8 and T_9 respectively were found to be 1248.63mg, 1101.51mg and 797.79mg; the average percentage growth recorded being 1451.23 (T_7), 1300.63 (T_8) and 956.85 % (T_9). The daily increments in growth obtained were 17.84mg, 15.74mg and 11.4mg for these treatments. Graphical representation of weight gain is given in Fig.5 and growth curve based on biweekly weight gain in Fig. 6.

Analysis of variance (Table 4) showed that the growth of shrimps was significantly different in various treatments.

Table 3. Growth of *P. monodon* fed on feeds containing various growth promoters at different levels

Treatment	Repli-cation	Av.initial weight (mg)	Av.Final weight (mg)	Gain in weight (mg)	Av. live weight gain (mg)	% weight gain	Av. % weight gain
T_0	1	81.62	850.50	768.88	783.71 ±28.52 3	942.02	927.48 ± 14.41
	2	81.35	840.00	758.65		932.58	
	3	90.72	914.33	823.61		907.85	
T_1	1	86.67	830.08	743.41	800.67 ± 40.51	857.75	931.27 ± 52.11
	2	85.42	916.00	830.58		972.35	
	3	85.92	913.96	828.04		963.73	
T_2	1	87.30	850.50	763.20	774.29 ± 54.84	874.23	918.82 ± 82.88
	2	81.77	928.08	846.31		1034.99	
	3	84.20	797.57	713.37		847.23	
T_3	1	85.50	920.50	835.0	769.74 ± 58.07	976.61	907.47 ± 90.99
	2	80.70	860.97	780.27		966.88	
	3	89.09	783.03	693.94		778.92	
T_4	1	88.64	1035.20	946.56	936.23 ± 101.00	1067.87	1058.47 ± 112.70
	2	88.49	1142.90	1054.41		1191.56	
	3	88.18	895.89	807.71		915.98	
T_5	1	87.76	1172.0	1084.24	1111.82 ± 26.06	1235.46	1246.03 ± 34.50
	2	88.72	1235.50	1146.78		1292.58	
	3	91.27	1195.70	1104.43		1210.07	
T_6	1	84.44	969.70	885.26	860.03 ±89.80	1048.39	996.60 ± 106.70
	2	87.36	1042.60	955.24		1093.45	
	3	87.22	826.82	739.60		847.97	
T_7	1	85.00	1288.45	1203.45	1248.63 ± 41.33	1415.82	1451.23 ± 48.85
	2	81.90	1321.02	1239.12		1512.97	
	3	85.47	1388.80	1303.33		1524.89	
T_8	1	85.23	1434.20	1348.97	1101.57 ±175.15	1582.74	1300.63 ± 199.52
	2	84.77	1071.80	987.03		1164.36	
	3	83.87	1052.40	968.53		1154.8	
T_9	1	82.25	909.62	827.37	797.79 ± 36.75	1005.92	956.85 ± 80.89
	2	88.51	834.50	745.99		842.83	
	3	80.23	900.04	820.0		1021.82	

Table 4. Analysis of variance of the data on growth of *P. monodon* fed on feeds containing various growth promoters at different levels

Source	S.S	D.F	M.S.S	F
Diets	819396.99	9	91044.11	9.87 *
Error	184532.75	20	9226.64	
Total	1003929.74	29		

Comparison of treatment means based on critical difference

Critical difference = 135.29

Treatments	T_3	T_2	T_0	T_9	T_1	T_6	T_4	T_8	T_5	T_7
Mean	769.74	774.29	783.71	797.79	800.67	860.03	936.23	1101.51	1111.82	1248.63

Underscored means are not significantly different

*Significant at 5% level

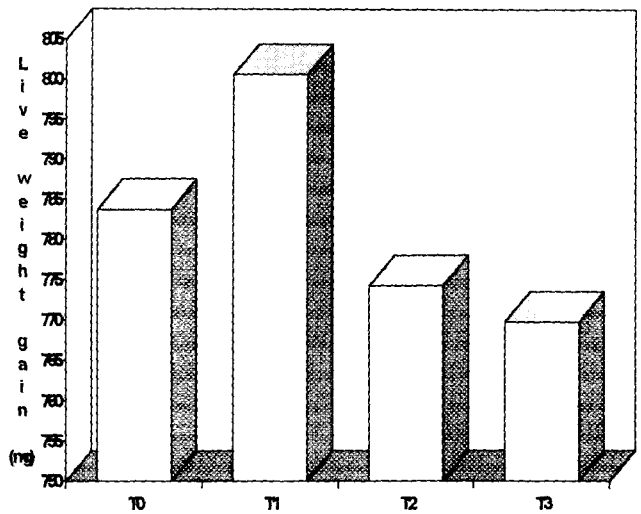


Fig. 1. Growth of *P. monodon* fed on feeds containing various levels of chitin.

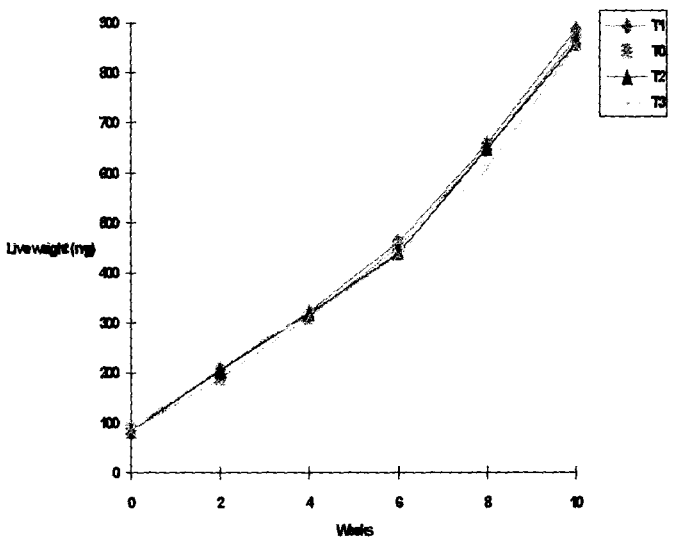


Fig. 2. Growth curve of *P. monodon* fed on feeds containing various levels of chitin.

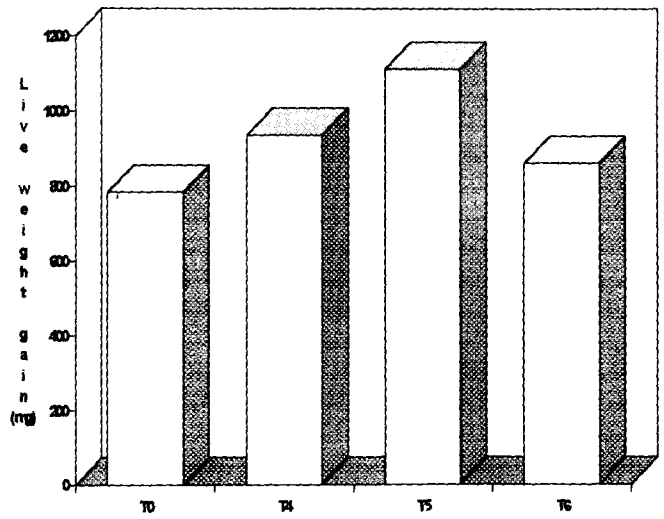


Fig. 3. Growth of *P. monodon* fed on feeds containing various levels of chitosan.

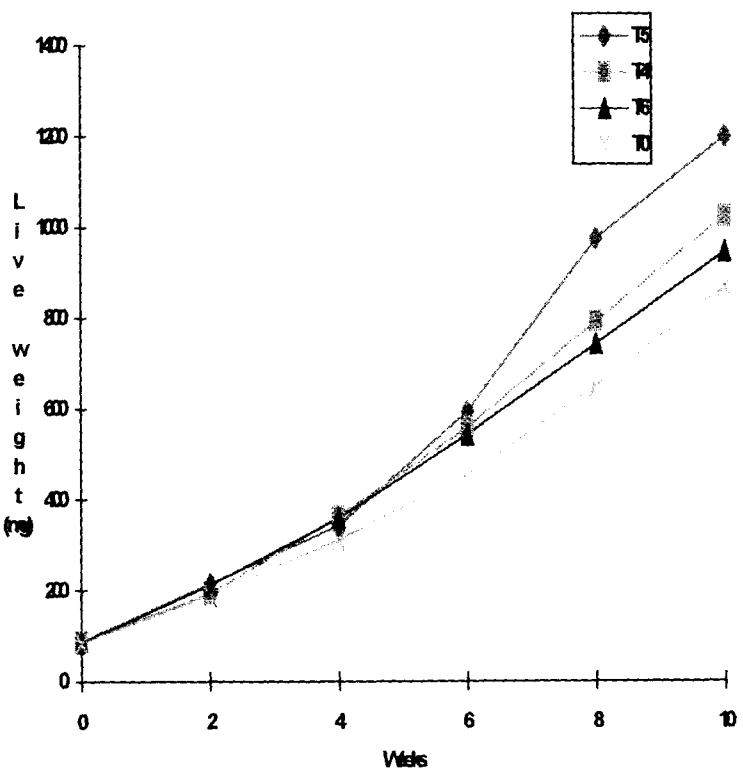


Fig. 4. Growth curve of *P. monodon* fed on feeds containing various levels of chitosan.

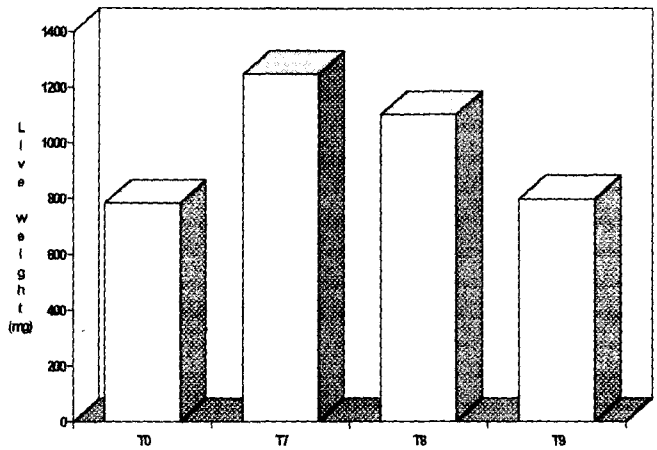


Fig. 5. Growth of *P. monodon* fed on feeds containing various levels of glucosamine.

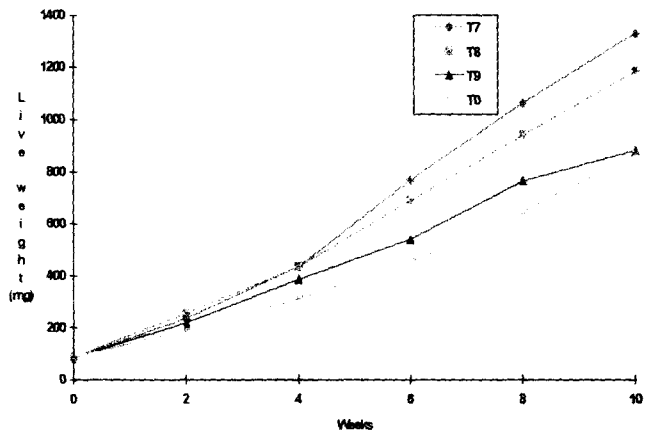


Fig. 6. Growth curve of *P. monodon* fed on feeds containing various levels of glucosamine.

The data regarding the growth in length of *P. monodon* juveniles fed on feeds containing various growth promoters at three levels are given in Table 5. The average gain in length recorded for different treatments being 2.09 (T_0), 2.23 (T_1), 2.23 (T_2),2.12 (T_3), 2.99 (T_4), 3.09 (T_5), 2.22 (T_6), 3.10 (T_7), 2.8 (T_8) and 2.63 (T_9) cm. Maximum length was achieved by shrimps in T_7, fed on glucosamine at 0.25g per 100g diet, followed by T_5 (0.5g chitosan / 100g diet), T_4 (0.25g chitosan / 100g diet) and T_8 (0.5g glucosamine / 100g diet). The percentage gain in length were 106.65, 110.65, 110.74 and 93.36 % for treatments T_7, T_5, T_4 and T_8 respectively. The growth in length of shrimps fed on different growth promoters at various levels are graphically represented in Fig. 7.

Analysis of variance (Table 6) showed that the growth in length of shrimps was significantly different between various treatments.

4.2.2. Specific growth rate.

The data on the specific growth are given in the Table 7. The SGR's of the shrimps fed on different levels of chitin were seen to be 3.33, 3.31 and 3.29 for T_1, T_2, and T_3 respectively; but none of these treatments were significantly different from the control (3.33). The SGR's of the *P. monodon* fed on chitosan were higher than the control being 3.49 (T_4), 3.62 (T_5) and 3.41 (T_6).Maximum specific growth rate of 3.94 was recorded for the diet containing glucosamine at 0.25g /100g diet. For the treatments T_8 and T_9 SGR value recorded were 3.75 and 3.36 respectively. The SGR of shrimps fed on different levels of chitin, chitosan and glucosamine are graphically represented in Fig 8.

Table 5. Growth in length of *P. monodon* fed on feeds containing various growth promoters at different levels

Treatment	Repli-cation	Av.initial length (cm)	Av.Final length (cm)	Gain in length (cm)	Av. gain in length (cm) ±SD	% gain in length	Av. % length gain
T_0	1	2.8	4.87	2.07	2.09 ± 0.09	73.93	74.69
	2	2.7	4.91	2.21		81.85	
	3	2.9	4.88	1.98		68.28	
T_1	1	2.8	5.06	2.26	2.23 ± 0.10	80.71	79.76
	2	2.8	4.90	2.10		75.00	
	3	2.8	5.14	2.34		83.57	
T_2	1	2.7	4.96	2.26	2.20 ± 0.04	83.70	77.15
	2	2.8	4.99	2.19		73.26	
	3	2.9	5.06	2.16		74.48	
T_3	1	2.9	5.02	2.12	2.12 ± 0.06	73.10	72.99
	2	2.9	5.09	2.19		75.52	
	3	2.9	4.94	2.04		70.34	
T_4	1	2.7	5.66	2.96	2.99 ± 0.10	109.62	110.74
	2	2.7	5.83	3.13		115.93	
	3	2.7	5.58	2.88		106.67	
T_5	1	2.9	5.90	3.0	3.09 ± 0.08	103.44	110.65
	2	2.7	5.89	3.19		118.15	
	3	2.8	5.89	3.09		110.36	
T_6	1	2.9	4.89	1.99	2.22 ± 0.19	68.62	76.55
	2	2.9	5.36	2.46		84.83	
	3	2.9	5.11	2.21		76.21	
T_7	1	3.0	5.98	2.98	3.10 ± 0.09	99.30	106.94
	2	2.8	6.01	3.21		114.64	
	3	2.9	6.00	3.10		106.89	
T_8	1	3.0	5.81	2.81	2.8 ± 0.07	93.67	93.36
	2	3.1	5.80	2.70		87.10	
	3	2.9	5.78	2.88		99.31	
T_9	1	2.8	5.53	2.73	2.63 ± 0.07	97.32	93.99
	2	2.8	5.40	2.60		92.86	
	3	2.8	5.37	2.57		91.79	

Table 6. Analysis of variance of the data on growth in length of *P. monodon* fed on feeds containing various growth promoters at different levels

Source	S.S	D.F	M.S.S	F
Diets	4.76	9	0.53	26.5 *
Error	0.29	20	0.02	
Total	5.053	29		

Comparison of treatment means based on critical difference

Critical difference = 0.20

Treatments	T_0	T_3	T_2	T_6	T_1	T_9	T_8	T_4	T_5	T_7
Means	2.09	2.12	2.20	2.22	2.23	2.63	2.8	2.99	3.09	3.10

Underscored means are not significantly different

*Significant at 5% level

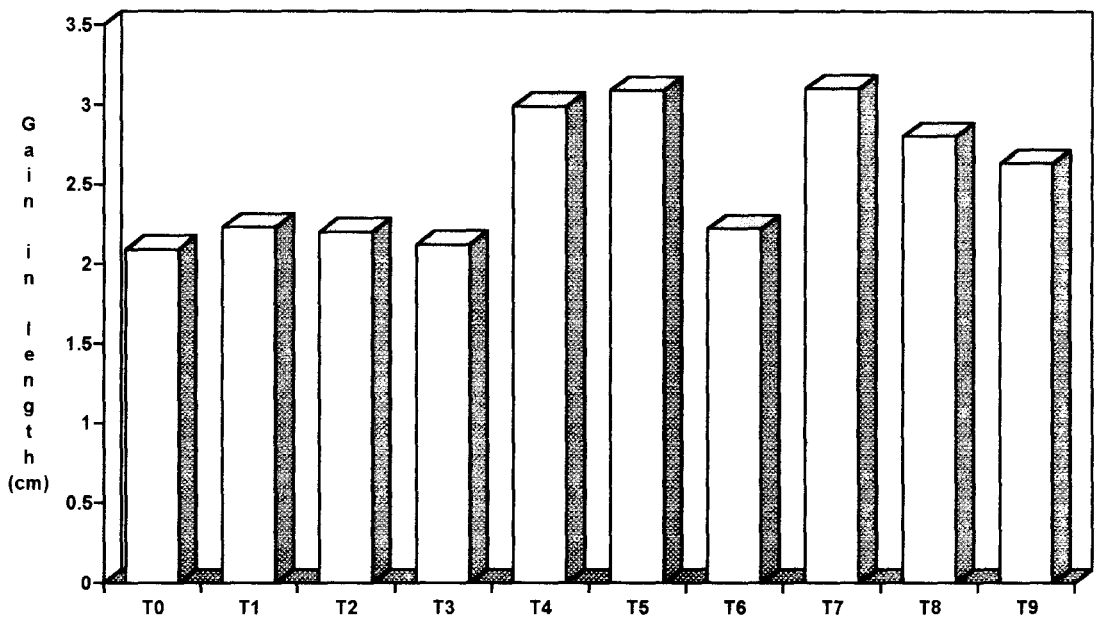


Fig. 7. Growth in length of *P. monodon* fed on feeds containing various levels of chitin, chitosan and glucosamine.

Table 7. Specific growth rate of *P. monodon* fed on feeds containing various growth promoters at different levels

Treatment	Repli-cation	Av.Initial weight (mg)	Av.Final weight (mg)	Specific growth rate	Mean ± S.D
T_0	1	81.62	850.50	3.35	3.33 ± 0.02
	2	81.35	840.00	3.34	
	3	90.72	914.33	3.30	
T_1	1	86.67	830.08	3.23	3.33 ± 0.07
	2	85.42	916.00	3.39	
	3	85.92	913.96	3.38	
T_2	1	87.30	850.50	3.25	3.31 ± 0.11
	2	81.77	928.08	3.47	
	3	84.20	797.57	3.21	
T_3	1	85.50	920.50	3.39	3.29 ± 0.13
	2	80.70	860.97	3.38	
	3	89.09	783.03	3.11	
T_4	1	88.64	1035.20	3.51	3.49 ± 0.14
	2	88.49	1142.90	3.65	
	3	88.18	895.89	3.31	
T_5	1	87.76	1172.0	3.70	3.62 ± 0.16
	2	88.72	1235.50	3.76	
	3	91.27	1195.70	3.39	
T_6	1	84.44	969.70	3.49	3.41 ± 0.15
	2	87.36	1042.60	3.54	
	3	87.22	826.82	3.21	
T_7	1	85.00	1288.45	3.88	3.94 ± 0.04
	2	81.90	1321.02	3.97	
	3	85.47	1388.80	3.98	
T_8	1	85.23	1434.20	4.03	3.75 ± 0.20
	2	84.77	1071.80	3.62	
	3	83.87	1052.40	3.61	
T_9	1	82.25	909.62	3.43	3.36 ± 0.11
	2	88.51	834.50	3.21	
	3	80.23	900.04	3.45	

Table 8. Analysis of variance of the data on specific growth rate of *P. monodon* fed on feeds containing various growth promoters at different levels

Source	S.S	D.F	M.S.S	F
Diets	1.301	9	0.1445	6.23 *
Error	0.464	20	0.0232	
Total	1.7654	29		

Comparison of treatment means based on critical difference

Critical difference = 0.22

Treatments	T_3	T_2	T_0	T_1	T_9	T_6	T_4	T_5	T_8	T_7
Mean	3.29	3.31	3.33	3.33	3.36	3.41	3.49	3.62	3.75	3.94

Underscored means are not significantly different

*Significant at 5% level

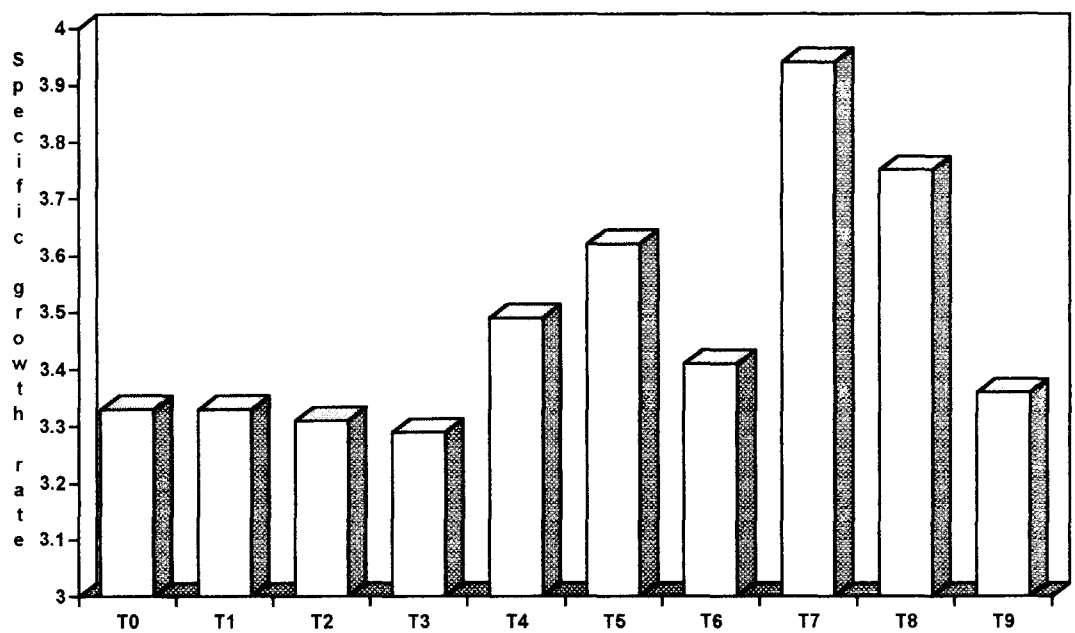


Fig. 8. Specific growth rate of *P. monodon* fed on feeds containing various levels of chitin, chitosan and glucosamine.

Analysis of variance of the data shows that there is significant difference between treatments (Table 8).

4.2.3. survival.

The percentage survival values of *P. monodon* juveniles in various treatments are given in Table 9. Highest average survival was obtained in treatment T_7 (83.3%) while the lowest value was recorded in the treatment T_0 (66.66%). Graphical representation of percentage survival values for various levels of chitin, chitosan and glucosamine are given in the Fig. 9. Analysis of variance of the data (Table 10) shows no significant difference between the treatments.

4.2.4. Food conversion ratio.

Food conversion ratio values of shrimp juveniles fed on various levels of the three growth promoters are given in Table 11. The mean FCR values ranged from 2.41 to 3.9 in various treatments.

The average FCR values in chitin based diet were 3.27, 3.66 and 3.78 for T_1, T_2 and T_3 respectively. None of these treatments were significantly different from that of control which recorded FCR of 3.61. However FCR of 3.13 and 2.65 in the diet Containing o.25 (T_4) and 0.5 (T_5) g chitosan per 100g diet exhibited significant difference from control while the FCR of the treatment T_6 (3.34) did not vary significantly. The FCR values of the diet with glucosamine as the growth promoter were 2.41, 2.67 and 3.9 for T_7, T_8 and T_9 respectively . Of these, FCR for treatments T_7 and T_8 were found to be significantly different from control. FCR of shrimp

T_7 and T_8 were found to be significantly different from control. FCR of shrimp receiving different level of chitin, chitosan and glucosamine are represented graphically in Fig. 10. Analysis of variance of FCR values shows significant difference between various treatments (Table 12).

4.2.5. Protein efficiency ratio.

The Protein efficiency ratio of various treatments are given in Table 13. The mean FCR ranged from 0.64 to 1.03. The PER values obtained for T_1, T_2, and T_3 containing various levels of chitin were 0.73, 0.68 and 0.66 while that of the control T_0, was 0.69. None of the values were significantly different from the control. Higher protein efficiency ratio was obtained in the diets containing chitosan as growth promoter; the PER values being 0.78, 0.94 and 0.75 for T_4, T_5 and T_6 respectively. However the maximum values were obtained in diets where glucosamine was incorporated at different levels. The values were seen to be 1.03, 0.94 and 0.64 for T_7, T_8 and T_9. The PER values of *P. monodon* juveniles fed on diets with various levels of chitin, chitosan and glucosamine are graphically represented in Fig. 11. Analysis of variance of the data shows significant difference among various treatments (Table 14).

Table 9. Percentage survival obtained in various treatments during the period of study to evaluate the efficiency of various growth promoters

Treatment	Replication	% Survival	Mean ± S.D
T_0	1	60	66.66 ±4.71
	2	70	
	3	70	
T_1	1	100	76.67 ±17.00
	2	70	
	3	60	
T_2	1	80	76.67 ±12.47
	2	90	
	3	60	
T_3	1	60	80.00 ±16.33
	2	80	
	3	100	
T_4	1	70	80.00 ±14.14
	2	70	
	3	100	
T_5	1	80	80.00 ±0
	2	80	
	3	80	
T_6	1	70	70.00 ±0
	2	70	
	3	70	
T-	1	80	83.30 ±4.71
	2	80	
	3	90	
T_8	1	70	70 ±8.16
	2	80	
	3	60	
T_9	1	80	70 ±8.16
	2	70	
	3	60	

Table 10. Analysis of variance of the data on survival of *P. monodon* fed on feeds containing various growth promoters at different levels

Source	S.S	D.F	M.S.S	F
Diets	880.00	9	97.78	0.60
Error	3266.67	20	163.33	
Total	4146.67	29		

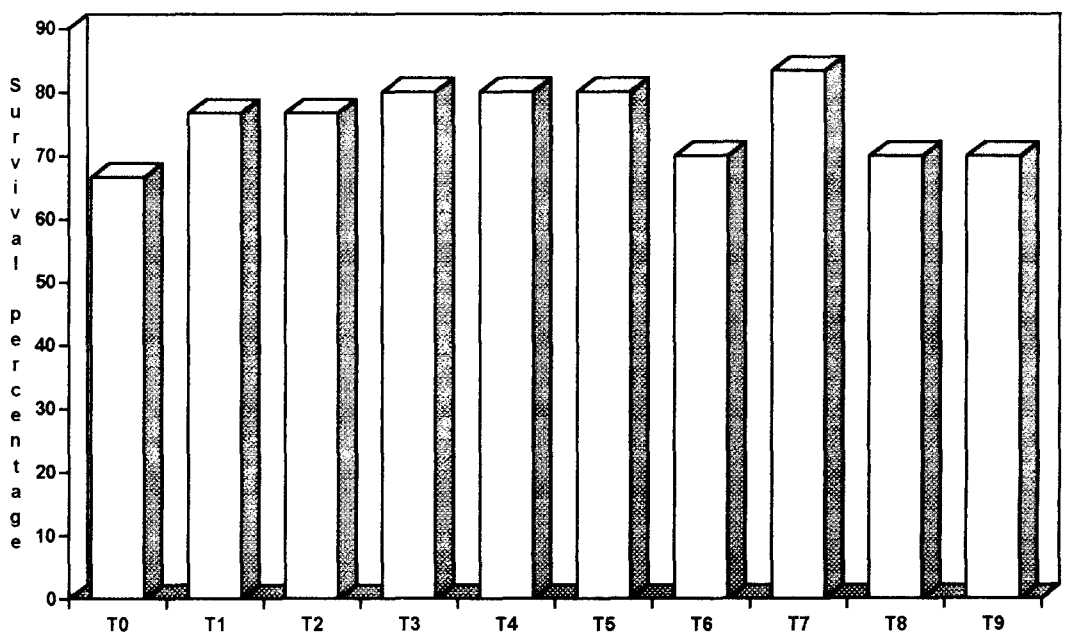


Fig. 9. Percentage survival of *P. monodon* fed on feeds containing various levels of chitin, chitosan and glucosamine.

4.2.6. Productive protein value.

The average initial body protein of shrimp juveniles was estimated as 13.22%., on a wet weight basis. Final body protein values have Shown that there is significant difference in protein deposition between in different treatments. Maximum body protein was seen in T_7 (15.6%) and minimum in T_3 (13.63%). PPV of various treatments are entered in Table 15.

Productive protein values for diets with different levels of chitin were 10.4 (T_1), 9.4 (T_2) and 8.97 (T_3) respectively . None of them were significantly different from the control group which had a PPV of 9.57. Productive protein values obtained for chitosan incorporated diet were 11.52 (T_4),14.41 (T_5) and 10.88 (T_6) respectively while those for diets containing glucosamine were found to be 16.45, 14.38 and 9.09 for T_7, T_8 and T_9. The PPV of the shrimps fed on different levels of chitin, chitosan and glucosamine are graphically represented in Fig. 12. Analysis of variance of the data shows significant difference between various treatments (Table 16).

4.3. Water quality parameters

4.3.1. Temperature.

The range of temperature in the experimental tanks during the study period are given in Table 17. Minimum temperature recorded was 26.0°C and maximum temperature recorded was 30.5°C. Weekly mean temperature values ranged from 26.77°C to 29.76°C.

Table 11. Food conversion ratio of *P. monodon* fed on feeds containing various growth promoters at different levels

Treatment	Repli-cation	Initial biomass (g)	Final biomass (g)	Growth increment (g)	Feed consumed (g)	FCR	Mean ± S.D
T_0	1	0.8162	7.003	6.1868	23.0564	3.73	3.61 ±0.11
	2	0.8135	6.651	5.8375	21.1844	3.63	
	3	0.9072	7.94	7.0328	24.4264	3.47	
T_1	1	0.8667	8.3008	7.43	23.9993	3.23	3.27 ±0.19
	2	0.8542	8.122	7.2678	25.52	3.51	
	3	0.8592	7.262	6.4028	22.20	3.06	
T_2	1	0.8730	7.904	7.031	27.823	3.96	3.66 ±0.32
	2	0.8177	8.7028	7.8851	25.4241	3.22	
	3	0.8420	7.4612	6.6192	25.2537	3.81	
T_3	1	0.8550	7.4945	6.6395	25.4616	3.83	3.78 ±0.04
	2	0.8070	7.5878	6.7808	25.6361	3.78	
	3	0.8909	7.8303	6.9394	25.928	3.74	
T_4	1	0.8864	9.0472	8.1832	25.7423	3.15	3.13 ±0.04
	2	0.8849	9.405	8.5201	26.2498	3.08	
	3	0.8818	8.9589	8.0771	25.4995	3.16	
T_5	1	0.8776	10.476	9.5984	26.1546	2.72	2.65 ±0.07
	2	0.8872	10.6743	9.7871	26.1624	2.67	
	3	0.9127	10.919	9.9992	25.5335	2.55	
T_6	1	0.8444	9.4229	8.5785	26.1155	3.04	3.34 ±0.30
	2	0.8736	8.9418	8.0682	26.1523	3.24	
	3	0.8722	7.9808	7.1086	26.6294	3.75	
T_7	1	0.85	11.9096	11.0596	27.3781	2.48	2.41 ±0.08
	2	0.819	11.7415	10.9225	26.6559	2.44	
	3	0.8547	13.2992	12.4445	28.6748	2.30	
T_8	1	0.8523	11.830	10.9777	27.4296	2.50	2.67 ±0.30
	2	0.8471	10.5992	9.7521	23.6249	2.42	
	3	0.8387	9.2695	8.4308	26.6617	3.09	
T_9	1	0.8225	8.2726	7.4501	26.5557	3.56	3.9 ±0.24
	2	0.8851	7.3165	6.4314	26.0271	4.05	
	3	0.8023	7.381	6.5787	26.9538	4.10	

Table 12. Analysis of variance of the data on food conversion ratio of *P. monodon* fed on feeds containing various growth promoters at different levels

.Source	S.S	D.F	M.S.S	F
Diets	7.338	9	0.815	13.59*
Error	1.199	20	0.06	
Total	8.537	29		

Comparison of treatment means based on critical difference

Critical difference = 0.35

Treatments	T_9	T_3	T_2	T_0	T_6	T_1	T_4	T_8	T_5	T_7
Mean	3.9	3.78	3.66	3.61	3.34	3.27	3.13	2.67	2.65	2.41

Underscored means are not significantly different

*Significant at 5% level

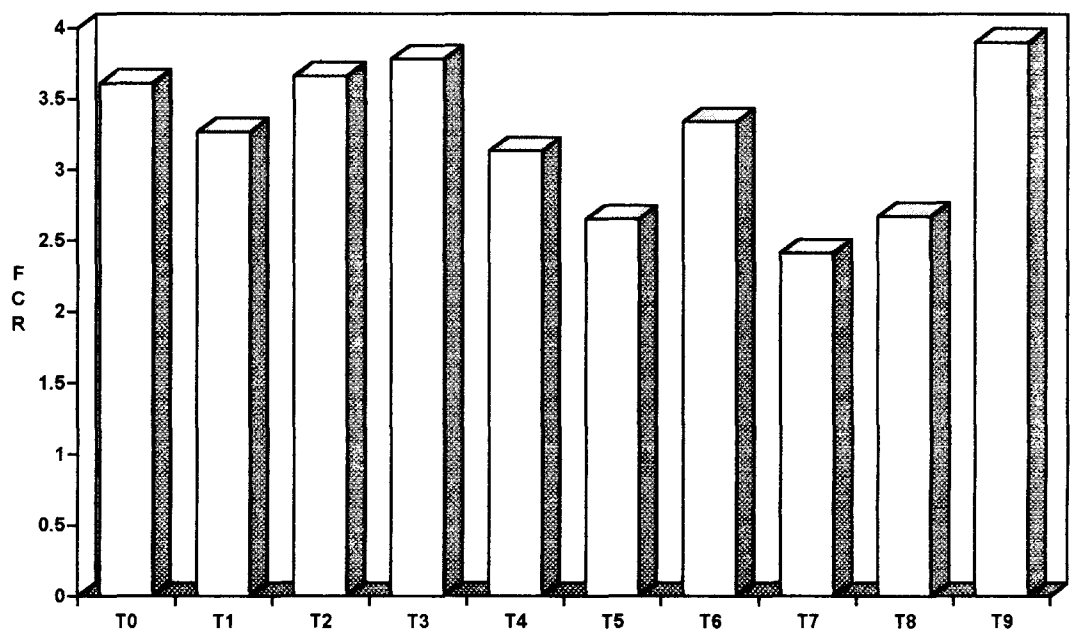


Fig. 10. Food conversion ratio of *P. monodon* fed on feeds containing various levels of chitin, chitosan and glucosamine.

Table 13. Protein efficiency ratio of P. monodon fed on feeds containing various growth promoters at different levels

Treatment	Repli-cation	Initial weight (g)	Final weight (g)	Av. live weight gain. (g)	Av. protein consumed (g)	PER	Mean ± S.D.
T_0	1	0.8162	7.003	6.1868	9.29	0.665	0.69 ±0.02
	2	0.8135	6.651	5.8375	8.53	0.685	
	3	0.9072	7.94	7.0328	9.98	0.704	
T_1	1	0.8667	8.3008	7.43	9.67	0.768	0.73 ±0.03
	2	0.8542	8.122	7.2678	10.28	0.707	
	3	0.8592	7.262	6.4028	8.94	0.716	
T_2	1	0.8730	7.904	7.031	11.36	0.619	0.68 ±0.06
	2	0.8177	8.7028	7.8851	10.24	0.77	
	3	0.8420	7.4616	6.6192	10.17	0.651	
T_3	1	0.8550	7.4945	6.6395	10.26	0.647	0.66 ±0.01
	2	0.8070	7.5878	6.7808	10.33	0.656	
	3	0.8909	7.8303	6.9394	10.45	0.664	
T_4	1	0.8864	9.0472	8.1832	11.03	0.742	0.78 ±0.03
	2	0.8849	9.405	8.5201	10.57	0.806	
	3	0.8818	8.9589	8.0771	10.27	0.786	
T_5	1	0.8776	10.476	9.5984	10.54	0.911	0.94 ±0.03
	2	0.8872	10.6743	9.7871	10.54	0.93	
	3	0.9127	10.919	9.9992	10.29.	0.972	
T_6	1	0.8444	9.4229	8.5785	10.52	0.815	0.75 ±0.06
	2	0.8736	8.9418	8.0682	10.54	0.765	
	3	0.8722	7.9808	7.1086	10.73	0.662	
T_7	1	0.8500	11.9096	11.0596	11.03	1.00	1.03 ±0.03
	2	0.8190	11.7415	10.9225	10.74	1.02	
	3	0.8547	13.2992	12.4445	11.55	1.08	
T_8	1	0.8523	11.830	10.9777	11.05	0.993	0.94 ±0.10
	2	0.8477	10.5992	9.7521	9.52	1.02	
	3	0.8387	9.2695	8.4308	10.50	0.802	
T_9	1	0.8225	8.2726	7.4501	10.70	0.696	0.64 ±0.04
	2	0.8851	7.3165	6.4314	10.49	0.613	
	3	0.8023	7.381	6.5787	10.86	0.605	

Table 14. Analysis of variance of the data on protein efficiency ratio of *P. monodon* fed on feeds containing various growth promoters at different levels

Source	S.S	D.F	M.S.S	F
Diets	0.516	9	0.0573	16.62*
Error	0.069	20	0.00345	
Total	0.585	29		

Comparison of treatment means based on critical difference

Critical difference = 0.08

Treatments	T_9	T_3	T_2	T_0	T_1	T_6	T_4	T_8	T_5	T_7
Mean	0.638	0.66	0.68	0.684	0.73	0.747	0.778	0.938	0.938	1.03

Underscored means are not significantly different

*Significant at 5% level

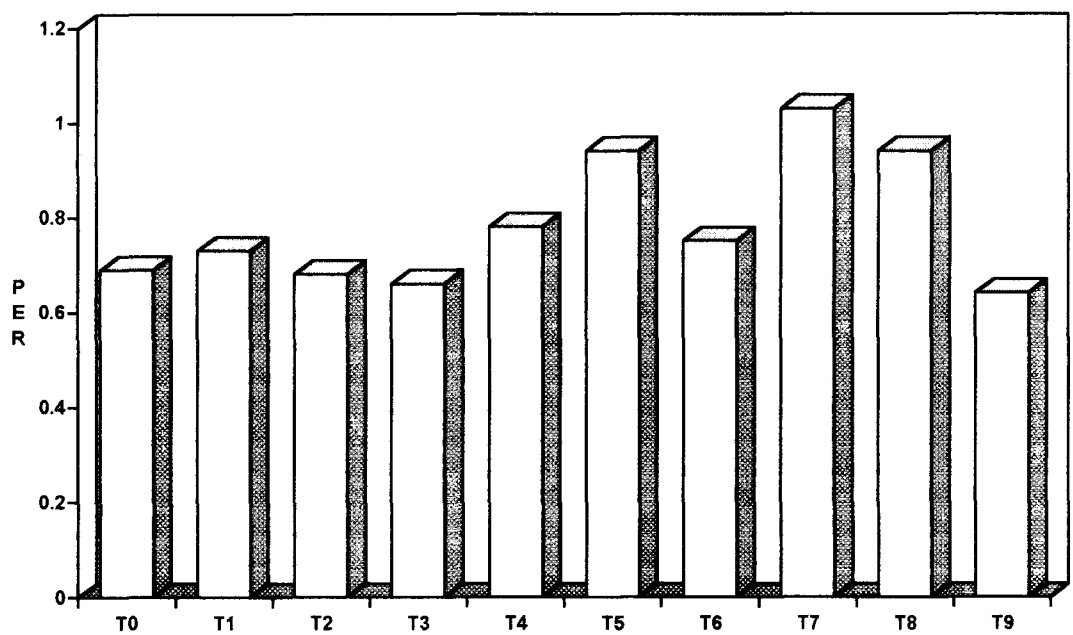


Fig. 11. Protein efficiency ratio of *P. monodon* fed on feeds containing various levels of chitin, chitosan and glucosamine.

Table 15. Productive protein value of *P. monodon* fed on feeds containing various growth promoters at different levels

Treatment	Replication	Initial weight (g)	Initial body protein (g)	Final weight (g)	Final body protein (g)	Gain in protein (g)	protein consumed (g)	PPV	Mean ± S.D
T_0	1	0.8162	0.108	7.003	0.972	0.864	9.29	9.30	9.57 ±0.22
	2	0.8135	0.108	6.651	0.923	0.815	8.53	9.56	
	3	0.9072	0.120	7.94	1.102	0.982	9.98	9.84	
T_1	1	0.8667	0.115	8.3008	1.173	1.058	9.67	10.94	10.40 ±0.39
	2	0.8542	0.113	8.122	1.148	1.035	10.28	10.06	
	3	0.8592	0.114	7.262	1.026	0.912	8.94	10.20	
T_2	1	0.8730	0.115	7.904	1.087	0.972	11.36	8.56	9.40 ±0.49
	2	0.8177	0.108	8.7028	1.200	1.092	10.24	10.66	
	3	0.8420	0.111	7.4612	1.025	0.914	10.17	8.99	
T_3	1	0.8550	0.113	7.4945	1.022	0.909	10.26	8.86	8.97 ±0.09
	2	0.8070	0.107	7.5878	1.034	0.927	10.33	8.97	
	3	0.8909	0.118	7.8303	1.067	0.949	1045	9.08	
T_4	1	0.8864	0.117	9.0472	1.334	1.217	11.03	11.03	11.52 ±0.35
	2	0.8849	0.117	9.405	1.364	1.247	10.57	11.80	
	3	0.8818	0.117	8.9589	1.321	1.204	10.27	11.72	
T_5	1	0.8776	0.166	10.476	1.611	1.495	10.54	14.18	14.41 ±0.55
	2	0.8872	0.177	10.6743	1.642	1.465	10.54	13.90	
	3	0.9127	0.121	10.919	1.680	1.559	10.29.	15.15	
T_6	1	0.8444	0.112	9.4229	1.350	1.238	10.52	12.14	10.88 ±1.07
	2	0.8736	0.116	8.9418	1.274	1.158	10.54	10.99	
	3	0.8722	0.115	7.9808	1.137	1.022	10.73	9.52	
T_7	1	0.8500	0.112	11.9096	1.876	1.764	11.03	15.99	16.45 ±0.51
	2	0.8190	0.108	11.7415	1.849	1.741	10.74	16.21	
	3	0.8547	0.113	13.2992	2.095	1.982	11.55	17.16	
T_8	1	0.8523	0.113	11.830	1.790	1.677	11.05	15.18	14.38 ±1.49
	2	0.8477	0.112	10.5992	1.604	1.492	9.52	15.67	
	3	0.8387	0.111	9.2695	1.402	1.291	10.50	12.30	
T_9	1	0.8225	0.109	8.2726	1.169	1.060	10.70	9.91	9.09 ±0.58
	2	0.8851	0.117	7.3165	1.033	0.916	10.49	8.73	
	3	0.8023	0.106	7.381	1.043	0.937	10.86	8.63	

Table 16. Analysis of variance of the data on productive protein value of *P. monodon* fed on feeds containing various growth promoters at different levels.

Source	S.S	D.F	M.S.S	F
Diets	194.2622	9	21.58	25.48*
Error	16.94	20	0.847	
Total	211.1974	29		

Comparison of treatment means based on critical difference

Critical difference = 1.3

Treatments	T_3	T_9	T_2	T_0	T_1	T_6	T_4	T_8	T_5	T_7
Mean	8.97	9.09	9.4	9.57	10.4	10.88	11.52	14.33	14.41	16.6

Underscored means are not significantly different

*Significant at 5% level

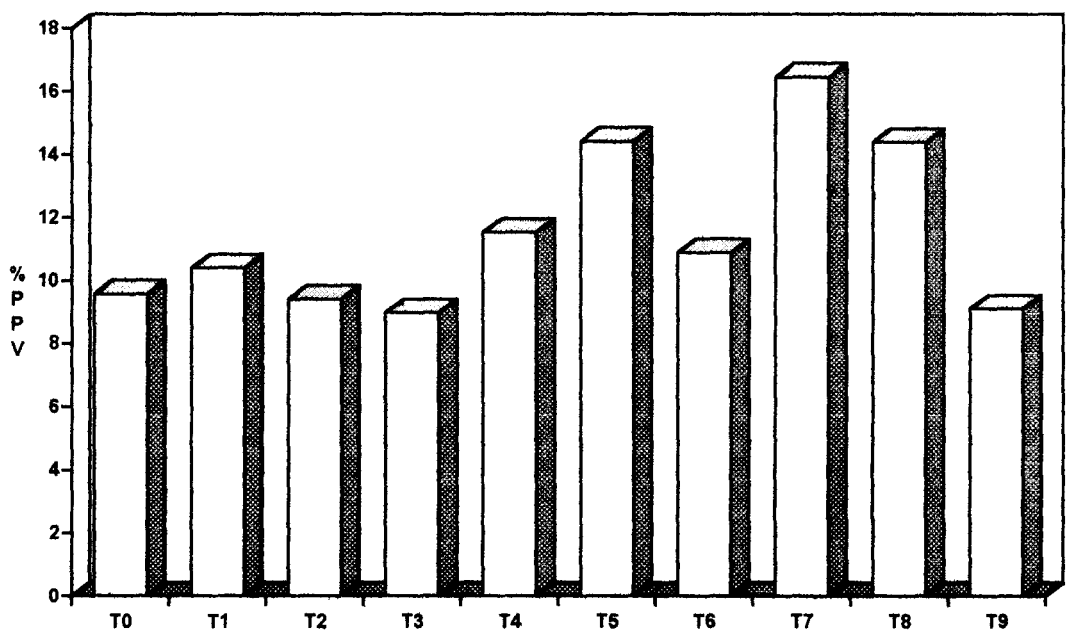


Fig. 12. Productive protein value of *P. monodon* fed on feeds containing various levels of chitin, chitosan and glucosamine.

4.3.2. pH.

Range of pH in the experimental tanks are given in the Table 18. Minimum pH observed was 7.6 while a maximum value of 9.0 was observed during evening hours. Weekly mean pH values ranged from 8.0 to 8.54.

4.3.3. Dissolved oxygen.

Range of dissolved oxygen in the experimental tanks are given in Table 19. A minimum of 6.18ppm was obtained during early morning hours while a high dissolved oxygen content of 8.32 was obtained during evening hours. Mean values ranged from 6.84 to 7.62ppm.

Table 17. Water temperature in experimental tanks during the study period.

Temperature	WEEKS									
	1	2	3	4	5	6	7	8	9	10
Mean	29.76	29.09	27.67	27.24	27.3	27.0	27.84	27.5	26.77	27.63
±SE	0.94	0.61	0.55	0.65	0.48	0.46	0.48	0.55	0.56	0.41
Range	29 - 30.5	28.3 - 30.5	26.8 - 28.5	26.5 -28.4	26.6 - 28.0	26.4 - 27.5	27.3 - 28.3	26.4- 28	26.0 - 27.5	27.2- 28.2

Table 18. pH of water in experimental tanks during the study period

pH	WEEKS									
	1	2	3	4	5	6	7	8	9	10
Mean	8.36	8.54	8.47	8.00	8.09	8.54	8.66	8.23	8.36	8.37
±SE	0.35	0.44	0.19	0.20	0.33	0.32	0.19	0.30	0.35	0.39
Range	8.0- 9.0	7.8- 9.0	8.2- 8.5	7.8- 8.2	7.6- 8.5	8.0- 9.0	8.5- 9.0	7.8- 8.5	8.0- 9.0	7.8- 8.8

Table 19. Dissolved oxygen in the experimental tanks during the study period

Dissolved Oxygen	WEEKS									
	1	2	3	4	5	6	7	8	9	10
Mean	6.84	7.26	7.46	6.85	7.53	6.82	7.39	7.62	7.26	7.59
±SE	0.73	0.83	0.68	0.47	0.38	0.38	0.60	0.47	0.25	0.52
Range	6.18- 8.24	6.30- 8.28	6.63- 8.32	6.43- 7.50	7.00- 8.16	6.34- 7.25	6.96- 8.28	7.12- 8.24	6.92- 7.50	7.08- 8.28

DISCUSSION

V. DISCUSSION

The effect of different levels of chitin, chitosan and glucosamine on the growth of *P. monodon* juveniles was evaluated. Different indices, viz, percentage growth, specific growth rate, survival, food conversion ratio, protein efficiency ratio and productive protein value were statistically analysed and are discussed below.

5.1. Proximate analysis of feed

The protein requirement of *P. monodon* has been identified to be 40 % with a diet containing shrimp meal, soyabean meal, casein, fish meal and squid meal as protein sources by Alava and Lim (1983). Bautista (1986) has reported 40-50 % protein requirement while Shiau *et al.* (1991a) worked out the protein requirement of *P. monodon* to be 40 % using a casein based diet. Shiau and Chou (1991) recommended 36-40 % protein level in the diet. Chen (1993) also reported 40 % requirement of protein by this species. Proximate analysis of basal diet used in the present study revealed that it contains 40.29 % protein which is the optimum value suggested by various workers.

The basal diet used in the present experiment contained 22.8 % carbohydrate. Bages and Sloane (1981) and Alava and Pascual (1987) obtained higher growth rate in *P. monodon* with a diet containing 20% carbohydrate.

Consequently Shiau and Peng (1991) who used 20 % carbohydrate together with 40 % protein obtained high growth rate. They observed that *P. monodon* may be able to use a relatively high content of carbohydrate in the diet. But Catacutan (1991) did not find any difference in growth of juvenile *P. monodon* fed isonitrogenous diets containing 5-35 % carbohydrate.

Akiyama (1989) recommended lipid levels of 6-7.5% in commercial shrimp diets, while Sheen and D' Abramo (1989) found similar level to be optimal for prawns as well. The lipid content of the basal diet used in the study also contained 5.66 % lipid.The fibre content of the experimental feed which was estimated to be 12.9% can compensate the growth of shrimps according to Venkataramiah *et al.* (1975). Fair *et al.* (1980) also stated that dietary fibre up to 30% does not appear to suppress growth of the giant fresh water prawn *Macrobrachium rosenbergii*. The ash content was worked out to be 11.62 % in the experimental feed. According to Forster and Gabbot (1971) shrimps can digest up to 30 % of ash fraction when the ash content was as high as 15 %.

5.2. water quality parameters

5.2.1. Temperature.

A temperature range of 28 ± 2 ^{0}C has been found to be optimum for *P. monodon* growth (Forster and Beard, 1974). Several workers have reported wide temperature tolerance for *P. monodon* (Liao, 1977; Sasai, 1981; Chen, 1985). Chakraborti *et al.* (1986) observed a temperature tolerance of 24-30.5^{0}C for this

species. The maximum temperature tolerance of *P. monodon* has been found to be 35^0C (Ravichandran *et al.*, 1982).The weekly range of temperature observed during the present experimental period was 26.77 ^{0}C to 29.7 ^{0}C. The values recorded are well within the optimum range suggested for the growth of *P. monodon*. The temperature fluctuation was gradual and was maintained uniformly through out the experimental period.

5.2.2. pH.

Subramanyam (1973) has observed that *P. monodon* require slightly alkaline pH, the optimum range being 8.1-8.5. Chen (1985) also suggested a similar pH range for its rearing. Chakraborti *et al.* (1986), however, obtained maximum growth rate at pH 8.4-8.7. A pH range of 7.3-8.5 has been suggested to be suitable for nursery rearing of *P. monodon* by Parado-Estepa *et al.* (1990), while Noor-Hamid *et al.* (1992) recommended a near neutral pH of 7.6-8.0 for faster growth. During the present experiment the weekly range in pH values in the tanks was from 8.0 to 8.6. These values conform to those obtained by aforesaid workers and lie within the optimum range.

5.2.3. Dissolved oxygen.

Studies made by Liao and Huang (1975) and Chen (1985) revealed that the oxygen consumption of post larvae of *P. monodon* decreased whenever dissolved oxygen fell below 3.8 and 4.0 ppm. Subramanyam (1973) and Chakraborti *et al.* (1985) reported that the dissolved oxygen below 2.5 ppm affected the growth and survival of *P. monodon*. Chakraborti *et al.* (1986) have suggested the optimum range

to be 6.8-7.6 ppm for *P. monodon* though they can tolerate dissolved oxygen as low as 4.8 ppm. The weekly dissolved oxygen values in the experimental tanks ranged from 6.82 to 7.62 ppm, since mild aeration was provided to the tanks. These values were found to be optimal for their growth.

5.2.4. Salinity.

In brackish water ponds in Philippines juvenile *P. monodon* grew about 50-100 g at 10-20 ppt salinity (Mochizuki, 1979) while in Indonesia Manik *et al.* (1979) recorded better growth and production of juveniles at 15-20 ppt. Sundararajan *et al.* (1979); Sebastian *et al.* (1980) and Ravichandran *et al.* (1982) also reported optimum growth of *P. monodon* at a salinity range of 4-20 ppt. Navas (1988) did not find any significant difference in growth of *P. monodon* post larvae reared at 4.5 and 15 ppt with those reared at 20 ppt. Accordingly the salinity in the experimental tanks for rearing *P. monodon* juveniles has been fixed as 20 ppt and maintained uniformly throughout the experimental period.

5.3. Growth

Results of the present study show that incorporation of chitin to the diet did not result in significant weight gain over the control, although the weight attained by juveniles fed with 0.25 g/ 100g chitin was superior to control. But enhancement of chitin level to 0.5 or 1 g/100 g diet resulted in a proportionate reduction in growth rate as evidenced by weight as well as length.

Kanazava *et al.* (1970) obtained superior growth rate in *P. japonicus* by incorporating chitin at 4 % level and glucosamine at 1.5% level in a purified diet. They obtained 63-72% higher growth rate by using a combination of both. Kitabayashi *et al.* (1971), however failed to get any growth enhancement with any level of incorporation of chitin in *P. japonicus*, while Venkataramiah *et al.* (1978) got higher growth in *P. aztecus* with a diet containing shrimp shell waste and attributed this to the presence of chitin in the shell waste. In 1986, Vaitheswaran and Ali while using partially demineralised prawn shell containing 43% chitin at 0.8 % level in the diet of *P. indicus* obtained higher weight gain over the control.

Akiyama *et al.* (1989) reported a reduction in the apparent digestibility of feed containing chitin and recommended a lower level of prawn shell in the shrimp diet. Clark *et al.* (1993) also investigated apparent digestibility of chitin in penaeids, and have opined that chitin digestion varies among different species. Fox (1993) who obtained poor growth rate with chitin, reported a low digestibility for chitin in *P. monodon*. He observed that synthesis of endogenous chitinase in the digestive gland of shrimps occur at a slow rate and that juvenile shrimps are able to digest only small amounts of dietary chitin in the absence of bacterially produced chitinase. The results obtained in the present experiment show a comparatively higher growth rate at 0.25g chitin / 100g diet indicating that the optimum level of chitin for *P. monodon* may be around 0.25g chitin / 100g diet or even lower and that shrimp juveniles do utilise chitin from a dietary source.

The growth rate of *P. monodon* in the present study shows that incorporation of chitosan at 0.25 g/ 100g and 0.5g/ 100g diet were significantly better than the control groups whereas at 1g/ 100g level the growth rate was not significant. The optimum level was found to be 0.5g chitosan / 100g diet, which was statistically significant from 0.25g/ 100g diet level of supplementation. Growth in length with different levels of chitosan have also shown the same trend. This observation indicates that shrimps may be able to utilise chitosan, which is none other than deacetylated chitin. Chitosan has been identified as a substrate for chitinases and chitobiase (Jauniaux, 1966). Fenton *et al.* (1978) also observed chitosanolytic microbes inhabiting soil and water, degrading both chitin and chitosan. The present study indicates the possibility of intestinal chitinolytic enzymes (mainly from an exogenous source) acting upon chitosan more efficiently than chitin. However it is seen that increasing levels of chitosan above 0.5g / 100g diet significantly reduces its activity. Kono *et al.* (1987) found that chitosan can combine with nutrients and impede digestion or absorption in fin fishes which had nil or poor chitosanase activity. But according to Fox (1993), the chitinolytic activity in juvenile shrimps is mainly derived from gut bacteria, which in turn, is less in shrimps and prawns in comparison to fin fishes. In the present experiment it was found that the feed is better utilised when chitosan was incorporated to the diet which clearly indicates chitosanase activity in the digestive tract of *P. monodon*.

When glucosamine was incorporated in the experimental diet, maximum growth rate was obtained at 0.25g /100g diet level followed by 0.5g/ 100g diet level

both being statistically significant from other treatments. However higher incorporation of glucosamine (1g/ 100g diet) did not result in significant weight gain. Gain in length have also shown a similar trend. Kanazava *et al.* (1970) obtained higher growth rate in *P. japonicus* with glucosamine incorporation at 1.5 % level together with chitin at 4% level whereas Kitabayashi *et al.* (1971) obtained superior growth with 0.53 % glucosamine in the diet of the same species. Higher weight gain in *P. indicus* was obtained by Vaitheswaran and Ali (1986) with a purified diet containing 0.8% glucosamine. Deshimaru and Kuroki (1974), however did not get any growth enhancement in *P. japonicus* with glucosamine.

5.4. Specific growth rate

Results of the present study show that chitin had no significant effect on the growth of *P. monodon* juveniles. Moreover increasing dietary levels of chitin even resulted in significant reduction in the specific growth rate of shrimps. Lindsay *et al.* (1984) obtained SGR values of 1.44, 1.43 and 0.93 in Rainbow trout (*Salmo gairdneri*) at 4,10, and 25 g chitin / 100g diet respectively; whereas Vaitheswaran and Ali (1986) obtained SGR values as high as 5.2 in *P. indicus* with 0.8 % partially demineralised prawn shell. But Fox (1993) did not find any significant difference between 4,8,12 or 16 % chitin supplemented diets with the control group in *P. monodon* although he noted that increasing chitin level slightly improved the SGR. Maximum SGR of 4.3 was obtained with 12 and 16 % supplemented diet. Thus increase in dietary level of chitin resulted in a concomitant reduction in the specific growth rate of fishes.

Incorporation of chitosan in the diet caused a significant reduction in the growth of the animal in the present study. Diet compounded with 0.5g chitosan /100g diet produced the highest specific growth rate of 3.62 though it was not significantly different from 0.25g/ 100g supplemented diet (3.49). However, chitosan at a level of 1g/ 100g diet was seen to check the growth rate since SGR at 1g chitosan / 100g diet was found to be 3.41. Kono *et al.* (1987) who tried chitosan as a growth enhancer in Red sea bream, Japanese eel and yellow tail at 10 % level obtained lower growth rate than control. This might be because these fishes may not have been able to utilise such a high level of chitosan for its growth. The SGR values obtained by them for the three species were 3.3, 0.72 and 2.69 respectively which were less than that recorded for the control groups. The result of the present experiment with *P. monodon* is not in compliance with that of Kono *et al.* (*loc cit.*). This may be probably due to the difference in the chitosanolytic activity between these species.

Incorporation of glucosamine at 0.25 and 0.5 g/ 100g diet were seen to enhance the SGR significantly compared to the 1g/ 100g supplementation or the control diet. However maximum growth rate was obtained with 0.25g/100g supplementation (3.94) which was not significantly different from 0.5g/ 100g supplemented diet, recording a specific growth rate of 3.75. Kanazava *et al.* (1970) obtained SGR values of 1.2- 1.5 with a purified diet containing 1.5% glucosamine in *P. japonicus*. However Vaitheswaran and Ali (1986) obtained SGR values as high as 5.3 with 0.8% glucosamine supplementation in a purified diet for *P. indicus*.

5.5. Survival

With the addition of chitin in the diet the percentage survival values of *P. monodon* in the present experiment were maximum (80%) at 1g/ 100g diet supplementation level in comparison to the other two treatments of chitin, both registered survival of 76.7% each. However there was no significant difference between the treatments.

Kanazava *et al.* (1970) obtained survival values as high as 95-100 % in *P. japonicus* with 4% chitin in the diet. Sick and Andrews (1973) and Balazs *et al.* (1973) have obtained high survival values in shrimps with shrimp shell supplementation in the diet while Pascual and Destajo (1978) did not observe any enhancement of growth or increase in survival of shrimps when fed on shrimp shell supplemented diet. Simpson (1981) who studied the effect of shrimp shell in the growth and survival of prawns observed that the presence of chitin does not contribute to higher survival rate. A high survival of 75 % in *P. indicus* was reported by Vaitheswaran and Ali (1986) when the shrimps were fed on feeds containing partially demineralised prawn shell. Fox (1993) obtained a maximum survival of 82 % with 4% chitin supplementation in the diet of *P. monodon* although on further addition of chitin survival rate was seen to be reduced.

Percentage survival values with chitosan as a growth promoter in the present study indicated that there is no significant difference between various treatments. Maximum survival of 80% was achieved at both 0.25 and 0.5 g/100g level while

further addition (1g/100g) reduced the survival rate to 70 %. However there was no significant difference between the treatments.

Incorporation of glucosamine to the diet have resulted in maximum survival rate of *P. monodon* in the present experiment. At 0.25g/100g, the percentage survival was 83.3 % while further addition resulted in a reduction in survival by 10% from the former. Kanazava *et al.* (1970) obtained survival values as high as 95-100% in *P. japonicus* with glucosamine at 1.5% level, while Kitabayashi *et al.* (1971) reported increased survival rate in the same species with glucosamine supplementation at 0.53% level. The survival values obtained by Vaitheswaran and Ali (1986) at 0.8% level in *P. indicus* is comparable with the percentage survival obtained in the present study with glucosamine at 0.5 and 1g/ 100g diet. Maximum survival was obtained at 0.25g glucosamine / 100g diet which has been identified to be the optimum level for incorporation in to the practical feeds of *P. monodon* in grow out system.

5.6. Food conversion ratio.

In the present study, when chitin was incorporated in the diet of *P. monodon* the FCR values ranged from 3.27-3.78. Chitin at 0.25g/ 100g diet resulted in significant reduction in the food conversion ratio (3.27) compared to the control group where the FCR was 3.61. With 0.5 and 1g chitin per 100g diet the FCR values obtained were 3.66 and 3.78. Food conversion ratio ranging between 2.27 and 3.02 were reported by Ahmad Ali (1982) and Ahamad Ali and Mohamad (1985) in *P. indicus* with feed containing shrimp shell waste. Lindsay *et al.* (1984) reported FCR of 0.89, 0.89 and 1.53 with diets containing 4, 10 and 25 % chitin in *Salmo*

gairdneri. But they found the least FCR of 0.87 in the control group. The FCR value obtained by Vaitheswaran and Ali (1986) with partially demineralised prawn shell in *P. indicus* was reported to be 2.18 which was statistically significant from the control in which the FCR was 2.84. Kono *et al.* (1987) reported high feed efficiency of 17.7, 29.8 and 21.2 % with feeds containing 10 % chitin in Red sea bream, Japanese eel and Yellow tail. These feed efficiency values were found to be superior than that obtained for control group or those fed 10 % chitosan or 10% cellulose. FCR as high as 6.21 in *P. monodon* was reported by Fox (1993) with a chitin containing diet.

All levels of chitosan were found to produce significant reduction in feed conversion rate in the present study. Lowest FCR of 2.65 was produced by 0.5g/ 100g diet supplementation of chitosan. This may be possible because of the ability of chitosan to combine with the nutrients of the feed thus preventing its leaching into the water. However Kono *et al.* (1987) reported poor feed efficiency in Red sea bream, Japanese eel and Yellow tail with 10 % chitosan supplementation. This may be due to difference in the chitosanolytic activity in various species. More over Kono *et al.* (*loc cit.*) have incorporated a higher level of chitosan to the feed and whether the level of chitosan had influenced the FCR is not clear.

Glucosamine incorporation at 0.25g / 100g diet was seen to have reducing the food conversion of shrimp juveniles to the minimum. Inclusion at 0.25 and 0.5g / 100g diet reduced the food conversion ratio to 2.41 and 2.67 respectively, but there was no significant difference between these two. However at 1g/ 100g level of incorporation FCR values were raised to 3.9 which was even more than the FCR

value obtained for the control (3.61). Vaitheswaran and Ali (1986) also obtained significant food conversion value of 2.05 in *P. indicus* when glucosamine was incorporated in the diet at 0.8% level.

5.7. Protein efficiency ratio

Appropriate and simple measures of the utilization of dietary protein by fish are protein efficiency ratio and productive protein value. Protein utilization depends essentially on fish species and size, environmental features, protein quality, level of dietary protein and utilizable dietary energy, kind of energy source and amount of feed (Steffens, 1981; Jauncey, 1982).

In the present study protein efficiency ratio of feed containing chitin at three levels were not found to be significant from the control. Protein efficiency ratio of feeds incorporated with 0.25, 0.5 and 1g chitin / 100g diet were 0.73, 0.68 and 0.66 respectively, while that of the control was 0.69. All treatments of chitosan have been found to be superior to the control group. Maximum PER of 0.94 was obtained with 0.5g chitosan / 100g diet, while 0.25 and 1g chitosan / 100g diet produced PER of 0.78 and 0.75 respectively, which were not significantly different from each other and from 0.25g chitin supplemented diet (0.73).

Feeds containing 0.25 and 0.5g glucosamine / 100g diet have been found to be superior to all other treatments tested. The PER of the former was 1.03 while that of the latter was 0.94. However 0.25g glucosamine / 100g diet was not statistically significant from 0.5g chitosan supplemented diet. Incorporation of glucosamine at still higher rates in the feed resulted in poor protein utilization, as evident from the

present experimental results where shrimp juveniles fed with 1g glucosamine / 100g diet supplemented diet had a PER of only 0.64 which was even lower than the control with PER of 0.69.

Colvin (1976 b) has obtained PER value as high as 0.95 in *P. indicus* while Sedgewick (1979) obtained PER value up to 0.90 in *P. merguiensis* with a feed based on *Mytilus edulis*. In 1992, Josekutty and Susheela obtained PER values as low as 0.817 for *P. monodon* when prawn shell was used as a protein source in the diet, while Anilkumar (1994) obtained a PER of 0.82 in *Macrobrachium rosenbergii* with the same protein source. PER values obtained with chitin as a growth promoter at three levels of incorporation were less than the value obtained in earlier studies with prawn shell waste as protein source in *Penaeus monodon* and *Macrobrachium rosenbergii*. However PER with chitosan at 0.5g/ 100g diet level and glucosamine at 0.25 and 0.5g/ 100g diet level were higher than the value obtained by Josekutty and Susheela (1992) with prawn shell in the same species. It was also comparable with PER obtained by Ahamad Ali (1982) with prawn shell waste in *P. indicus* and with that of Boby and Susheela (1996) with purified diet where oxytetracycline was incorporated as a growth promoter in *Macrobrachium rosenbergii*.

It may be presumed that *P. monodon* might be deriving a portion of its energy requirement from substances like chitin, chitosan or glucosamine at low levels of incorporation in the diet. However, higher levels of incorporation do not seem to have any influence on growth since the activity of the chitinolytic enzymes might be lower compared to adult shrimps or fin fishes. Fin fishes seem to be efficient utilizers

of dietary protein when compared to shell fishes since values as high as 2.7 (Steffens and Albrecht, 1979) and 3.3 (Takeuchi *et al.*, 1978 b) have been reported for Rainbow trout *Salmo gairdneri*. Degani *et al.* (1989) have also reported higher PER values in *Clarius gariepinus*.

5.8. Productive protein value

Protein utilization by fishes can also be expressed in terms of productive protein value. It has been used as an index of protein utilization (Steffens and Albrecht, 1975; Reinitz *et al.* 1978; Steffens, 1981; Degani *et* al., 1989). Very high PPV values of 34-39 have been obtained in *Salmo gairdneri* (Steffens and Albrecht, 1975; Cho *et al.* 1976; Reinitz *et* al. , 1978). Steffens (1981) has reported PPV as high as 30.3 in *Salmo gairdneri* and 23.2 in *Cyprinus carpio*. Takeuchi *et al.* (1978 b) obtained PPV of 51 in *Salmo gairdneri* while Degani *et al.* (1989) reported very high PPV in *Clarius gariepinus*. But studies on productive protein value of crustaceans especially of prawns and shrimps are scarce (James *et al.* 1990; Boby and Susheela, 1996).

In the present study with three levels of chitin incorporation in the feed the PPV obtained were not significantly different from the control group. PPV at 0.25g chitin / 100g diet level (10.4) was slightly higher than the control (9.57) even though they were not significantly different from each other. At higher levels of chitin incorporation (0.5 and 1g/ 100g diet) the protein retention was seen to be lower than the control.

All levels of chitosan supplementation in *P. monodon* showed significantly higher PPV than the control. Maximum protein retention was at 0.5g/ 100g diet level (14.41) followed by 0.25g/ 100g diet (11.52) and 1g/ 100g diet level (10.88). The latter two values (11.52 and 10.88) were found to be significantly different from the former (14.41).

Inclusion of glucosamine at 0.25g/ 100g diet gave the highest PPV (16.6) followed by glucosamine at 0.5g/ 100g diet level (14.38). However higher rate of incorporation resulted in lower performance than the control diet.

James *et al.* (1990) reported PPV of 6.67 for *Spirulina* based test diet and 9.42 for casein based control diet for *M. rosenbergii* post larvae. Boby and Susheela (1996) in their studies on the use of growth promoters in *M. rosenbergii* post larvae obtained a PPV of 13.48 when 10 ppm oxytetracycline was incorporated in a casein based purified diet; while the PPV of control diet was 8.99. The result of the present study indicates that glucosamine at 0.25 and 0.5g/ 100g diet and chitosan at 0.5g/ 100g diet are superior to other treatments in *P. monodon* in terms of protein deposition. Hence it may be summarised that efficient protein sparing action by these substances at specific levels of incorporation enabling faster growth of the animal.

The results of the present study indicates that chitin does not have a highly positive growth promoting role in *P. monodon* though small level of incorporation can enhance the growth slightly. Low levels of chitosan (0.5g /100g diet) is found to be very effective in promoting growth and feed conversion while high levels (1g/ 100g diet) do not produce such growth effect. Glucosamine at 0.25g/ 100g diet

seems superior to all other treatments based on growth, food conversion, PER and PPV. Very high levels of all growth promoters used in the present study were seen to lessen the efficiency and quality of feed thus reducing the growth rate. This may be due to the low chitinase activity in *P. monodon* juveniles as concluded by Fox (1993).

SUMMARY

VI. SUMMARY

The present experiment has been carried out to find out the comparative efficiency of chitin, chitosan and glucosamine as growth promoters in *Penaeus monodon* juveniles.

1. Each growth promoter was incorporated at three levels viz. 0.25, 0.5 and 1.0g per 100g diet, in order to find out the optimum level of each.

2. The growth promoters were incorporated into a practical diet based on soyflour-clam meal containing on an average 40.29% crude protein , 5.66 % crude fat and 22.93% carbohydrate.

3. The test diets prepared for the experiment were control diet To without incorporation of any growth promoter, feed containing 0.25, 0.5 and 1g chitin per 100g diet designated as T1, T2 and T3, feeds with similar levels of chitosan designated as T4, T5 and T6, and feed containing glucosamine at the same three levels designated as T7, T8 and T9.

4. The duration of the experiment was 70 days. Various parameters like growth, specific growth rate, survival, food conversion ratio, protein efficiency ratio and productive protein value were worked out in order to find out the comparative efficiency of each of the growth promoter.

5. The results showed that incorporation of chitin does not have any significant growth promoting effect in *P. monodon*, though the growth obtained

with feed containing 0.25g chitin per 100g diet was slightly more than that of control. Shrimps fed on all levels of chitosan have shown better growth than the control group. The weight gain of *P. monodon* juveniles fed on feeds containing glucosamine at 0.25 and 0.5g per 100g diet were superior so far as growth parameters were concerned; but increased levels of glucosamine had a negative effect on growth of shrimps. Growth in length has shown a similar trend in all cases.

6. The specific growth rates of shrimps fed on different levels of chitin were seen to be 3.33, 3.31 and 3.29 for T1, T2 and T3 respectively, none of which were significantly different from the control (3.33). The SGR 's of shrimps fed on diets containing chitosan were 3.49, 3.62 and 3.41 respectively for T4, T5 and T6. The treatment T5 was seen to be significantly different from the control group. Incorporation of glucosamine also resulted in an improved SGR in shrimps. Addition of 0.25 and 0.5g of glucosamine per 100g diet has produced the highest specific growth rate (3.94 and 3.75 respectively) while still higher inclusion resulted in lowering of SGR.

7. No significant difference was obtained in the percentage survival values when chitin was incorporated into the diet. Maximum survival of 80% was obtained at 1g chitin per 100g diet while the other two treatments of chitin had a survival rate of only 76.7% each, while that for the control group was 66. 7%. There was no significant difference in survival values between various treatments of chitosan too. Maximum survival of 80% was obtained at both 0.25 and 0.5g per 100g diet level while further addition (1g per 100g diet) reduced the survival rate to

70%. Incorporation of glucosamine at 0.25g per 100g diet resulted in maximum survival of 83 .3% in *P. monodon* in the present experiment. Further addition resulted in a reduction of 13% in survival rate from the former treatments

8. Chitin at 0.25g per 100g diet resulted in significant reduction in the food conversion ratio (3.27) compared to the control group where the FCR was 3.61. With 0.5 and 1g chitin per 100g diet the FCR values obtained were 3.66 and 3.78, both being not significantly different from the control. However FCR of 3.13 and 2.65 obtained in diets containing 0.25 (T4) and) 0.5g (T5) chitosan per 100g diet exhibited significant difference with control but the FCR value of 3.34 in treatment T6 (1g chitosan per 100g diet) did not vary significantly . FCR values of diets with glucosamine as the growth promoter were 2.41, 2.67 and 3.9 for T7, T8 and T9 respectively, the former two being significantly different from the control group.

9. Protein efficiency ratios of feed containing chitin at three levels were not found to vary significantly from the control. The PER of feeds incorporated with 0.25, 0.5 and 1g chitin per 100g diet were 0.73, 0.68 and 0.66 respectively while that of the control was 0.69. All levels of chitosan have been found to be superior to the control group, but maximum PER of 0.94 was obtained with 0.5g per 100g diet. Feeds containing 0.25 and 0.5g glucosamine per 100g diet were found to be superior to all other treatments tested. The PER values obtained for these treatments were 1.03 and 0.94 respectively while that for T9 with 1g per 100g diet was only 0.64 which was even lower than the control.

10. Chitin incorporation in the feed at 3 levels gave productive protein values of 10.4, 9.4 and 9.0 respectively for treatments T1, T2 and T3 while for the control it was 9.6. None of these treatments were significantly different from the control group. All levels of chitosan supplementation have shown significantly higher PPV than the control. Maximum protein retention was obtained at 0.5g chitosan per 100g diet level (14.41) followed by 0.25g (11.52) and 1g per 100g diet level (10.88) ; the former being statistically significant from the latter two. Inclusion of glucosamine at 0.25g per 100g diet gave the highest PPV (16.6) followed by glucosamine at 0.5g per 100g level (14.38) both being statistically significant from the control group. However higher rate of incorporation resulted in poor performance (9.09) than the control diet.

VII. REFERENCES

Agung, S., Hoxey, M. J., Kailis, S. G. and Evan, L. H. 1995. Investigation of alternate protein sources in practical diets for juvenile shrimp *Penaeus monodon*. *Aquaculture*, **134** : 313-323.

Ahamad Ali, S. 1982. Relative efficiencies of pelleted feeds compounded with different animal proteins and the effect of protein level on the growth of *Penaeus indicus*. *Proc. Symp.Coastal Aquaculture Mar. Biol. Assn India.*, **1**: 321-328.

Ahamad Ali, S. 1995. Feed addition for growth promotion in shrimp. *Fishing chimes.*, **15(3)** : 21-23.

Ahamad Ali, S. and Mohamed, K. H. 1985. Utilization of prawn waste and mantis shrimp for compounding feeds for the culture of penaeid prawns. *Proc. Symp. Harvest and Post Harvest Technology of Fish., Soc. Fish. Technologists.* : 615-618.

Ahamad Ali, S. and Sivadas, M. G. 1983. Compounded feeds for post larval rearing of marine prawns. *Proc. Nat. Symp. Shrimp. Seed Production and Hatchery Management*, Cochin, P. 159.

Ahamed, T. J. and Matty, A. J. 1989. The effect of feeding antibiotics on growth and body composition of carp (*Cyprinus carpio*). *Aquaculture*, **77** : 211-220.

*Akiyama, D. M. 1989. The use of soybean meal to replace white fish meal in commercially processed *Peneus monodon* feeds in Taiwan. *J. World. Aquacul. Soc.*, **20(1)**: 11A.

*Akiyama, D., Brocks, J. and Haley, S. 1982. Isopathic muscle necrosis in the cultured fresh water prawn (*Macrobrachium rosenbergii*). *Vet. Med. Small. Ani. Clin.*, July. 1119.

Akiyama, D. M., Coelho, S. R., Lawrence, A. L. and Robinson, E. H. 1989. Apparent digestibility of feed stuffs by the marine shrimp *Penaeus vannamei* BOONE. *Nippon Suisan Gakkaishi*, **55(1)** : 91-98.

Akiyama, D. M., Dominy, W. G. and Lawrence, A. L. 1991. Penaeid shrimp nutrition for the commercial feed industry. *Marine Shrimp Culture, Principles and Practices.* Fast and Lester (eds.). Elsevier Science Publications, Amsterdam, Netherlands, P. 535-568.

Alava, V. R. and Lim, C. 1983. The quantitative dietary protein requirements of *Penaeus monodon* juveniles in a controlled environment. *Aquaculture*, **30** : 53-61.

Alava, V. R. and Pascual, F. P. 1987. Carbohydrate requirements of *Penaeus monodon* (Fabricius) juveniles. *Aquaculture*, **61** : 211-217.

Allahpichay, I. and Shimizu, C. 1984 a. Supplemental effect of the whole body krill meal and the non muscle krill meal of *Euphausia superba* in fish diet. *Bull. Jap. Soc. Sci. Fish.*, **50(5)** : 815-820.

Allahpichay, I. and Shimizu, C. 1984 b. Extraction of growth promoting fractions from non-muscle krill meal of *Euphausia superba* and its effect on fish growth. *Bull. Jap. Soc. Sci. Fish.*, **50(5)** : 821-826.

Andrews, J.W., Sick, L.V. and Baptist, G.L. 1972. Influence of dietary protein and energy levels on growth and survival of Penaeid shrimp. *Aquaculture*, **1**: 341-347.

Anicic, I., Treer, T. and Safner, R. 1995. The influence of nutritive antibiotic on the production results in 1-year old carps (*Cyprinus carpio*) fingerlings. *Aquaculture*, **129** : 151 (Abstract).

Anilkumar, P. 1994. Evaluation of different protein sources in the formulated feed for *Macrobrachium rosenbergii* (De Man) juveniles. M.F.Sc thesis, Kerala Agri. Uni., P. 141.

Anon. 1990. A drug to boost shrimp growth. *Fish Farming International*, **17(3)**.

Anon. 1991. Chitin and chitosan. *Shrimp waste Utilization*. INFOFISH Technical hand book 4, P. 20.

Antiporda, J. L. 1986. Preliminary studies on the effect of Methyl testosterone on *Macrobrachium rosenbergii* juveniles. *NACA, Bangkok (Thailand) NACA / WP / 86 / 46.*, P. 21.

AOAC. 1975. Official methods of analysis. Association of official agricultural chemists. Washington D.C., 12th Ed., P. 1094.

*AQUACOP, Arzel, J. and Cuzon, G. 1989. Digestibility study on 3 different penaeid species fed on some protein sources. *J. World Maricul. Soc.*, **20(1)** : 12A.

AQUACOP, !977. Study of nutrition requirements and growth of *Penaeus merguiensis* in tanks by means of purified and artificial diets. *Proc. World. Mari. Soc.*, **9**: 225-234.

Ashraf, M., Meade, T. L 1993. Effect of triiodo thyronine (T_3) and 17-α methyl testosterone on growth and body composition of chinook salmon (*Oncorhynchus tshawytscha*). *Pak. J. Zool.*, **25(4)** : 299-302.

Bages, M. and Sloane, L. 1981. Effects of dietary protein and starch levels on growth and survival of *Penaeus monodon* (Fabricius) post larvae. *Aquaculture,* **25** : 117-128.

Balazs, G. H. 1973. Preliminary studies on the preparation and feeding of crustacean diets. *Aquaculture*, **2** : 369-377.

Balazs, G. H., Ross, E., Brooks, C. C. and Fujimura, T. 1974 b. Effect of protein source and level on growth of the captive fresh water prawn *Macrobrachium rosenbergii*. *Proc. World. Mari.Soc.*, **5** : 1-14.

Bautista, M. M. 1986. The response of *Penaeus monodon* juveniles to varying protein / energy ratios in test diets. *Aquaculture,* **53** : 229-242.S

*Berkeley, R. L. W. 1978. Chitinolytic and chitosanolytic microorganisms and the potential biodeterioration problems in the commercial application of chitin and its derivatives. In. Muzarelli, R. A. and Pariser, E. R. (eds.). *Proc. First International Conf. chitin/chitosan.* Boston, April 11-13, 1977 : 554-561.

Boby Ignatius and Susheela Jose. 1996. Use of selected growth promoters in the larval rearing of the giant fresh water prawn *Macrobrachium rosenbergii*. In. Iyengar, P. K. (ed.) *Proc. Eight Kerala Science Congress*, Kochi : 536-539.

Boddeke, R. 1983. Survival strategies of penaeid shrimps and their significance for shrimp culture. *Proc. First. Internat. Biennial conf. Warmwater Aquacul. Crustacea.* Laic, Hawaii, **1** : 514-523.

Boettcher, V. 1985. Experimental rearing of fry of carp (*Cyprinus carpio*) and Silver carp (*Hypophthalmichthys molitrix*) with variants of cyprinid starter feed. *CSF. A. Fischereiforschung,* **24(2)** : 7-12.

Bogut, I., Opacak, A. and Stevic, I. 1995. The influence of polyzymes added to the food on the growth of carp fingerlings (*Cyprinus carpio*). *Aquaculture*, **129**: 152 (Abstract).

Boyd, C.E. 1979. *Water quality in warm water fish ponds.* Auburn University Agricultural Experiment Station, Auburn, Alabama., P. 359.

Campbell, K.C. 1973a. Culture of aquatic animals. U.K. PATENT, 1, 304, 513.

Campbell, K.C. 1973b. Method of feeding chitinous meal to crustacea.U.S. patent, 3, 733, 204.

Carr, W. E. S. and Thompson, H. W. 1983. Adenosine-5'-monophosphate, an internal regulatory agent is a potent chemoattractant for marine shrimp. *J. Comp. Physiol.*, **153** : 47-53.

Catacutan, M. R. 1991. Apparent digestibility of diets with various carbohydrate levels and the growth response of *Penaeus monodon*. *Aquaculture*, **95** : 89-90.

Chakraborti, R. K., Holder, D. D., Das, N. K., Mandal, S. K. and Bhowmik, M. L. 1986. Growth of *Penaeus monodon* Fabricius under different environmental condition. *Aquaculture*, **51** : 189-194.

Chakraborti, R. K., Ravichandran, P., Holder, D. D.,Mandal, S. K. and Sanfui, D. 1985. Some physicochemical characteristics of Kakdwip brackish water ponds and their influence on the survival, growth and production of *Penaeus monodon* (Fabricius). *Ind. J. Fish.*, **32(2)** : 224-235.

Chandramohan, D. and Thomas, I. 1984. Studies on chitinase activity and chitinoclastic bacteria in sediments, fishes and prawns. *Proc. Symp. Coastal. Aquaculture. Mar. Biol. Assn. India.*, **3** : 839-845.

Chaudhary, M. A., Lone, K. P. and Matty, A. J. 1989. Oral administration of thyroxine and triiodothyronine on growth and food conversion of *Oreochromis mossambicus* and *Cyprinus carpio*. *Pak. J. Zool.*, **21(4)** : 307-310.

Chen, H. C. 1985. Water quality criteria for farming the grass shrimp *Penaeus monodon*. In. Taki, Y., Primavera, J. H. and Llobreva, J. A. (eds). *Proc. First. Internat. Conf. on the Culture of Penaeid prawns / shrimps*. SEAFDEC, P. 165 .

Chen, H. Y. and Lin, H. F. 1990. The effect of exogenous digestive enzymes on the growth of early *Penaeus monodon*. In. Hirano, R. and Hanyu, I. (eds.) *Proc. The Second Asian Fish. Forum.*, Asian Fisheries Society, Manila, Philippines : 349-352.

Chen, H.Y. 1993. Recent advances in nutrition of *Penaeus monodon*. *J. World. Aquacul. Soc.*, **24** : 231-240.

Cho, C. Y., Slinger, S. J. and Bayley, H. S. 1976. Influence of level and type of dietary protein and of level of feeding on food utilization by rainbow trout. *J. Nutri.*, **106** : 1547-1556.

Chua, T. E and Teng, S. K 1980. Economic production of estuary grouper *E. salmoides* Maxwell, reared in floating net cages. *Aquaculture*, **20** : 187-228.

Chuang, J. L., Lee, M. F. and Lenn, J. S. 1985. Comparison of digestive enzyme activities of five species of shrimp cultured in Taiwan. *J. Fish. Soc. Taiwan.*, **12(2)** : 43-53.

*Ciapara, H. I., Brown, J. H and Jauncey. 1989. Effect of OTC and sulphamethazine on weight gain and survival of *Penaeus monodon* under stress. Institute of Aquaculture, Sterling, Scotland FK9. 4Ln, U.K.

Clark, D. J., Lawrence, A. L. and Swakon, D. H. D. 1993. Apparent chitin digestibility in penaeid shrimp. *Aquaculture*, **109** : 51-57.

Clark, J., Quayle, K. A., Mac Donald, N. L. and Stark, J. R. 1988. Metabolism in marine flat fish- V- chitinolytic activities in Dover sole, *Solea solea. Comp. Biochem. Physiol.*, **90 B (2)** : 379-384.

Colvin, P. M. 1976 a. Nutritional studies on penaeid prawn : Protein requirements in compounded diets for juvenile *P. indicus. Aquaculture*, **7** : 315-326.

Colvin, P. M. 1976 b. The effect of selected seed oils on the fatty acid composition and growth of *Penaeus indicus. Aquaculture*, **8** : 81-89.

Corliss, J. P., Lighter, D. and Zein Eldin, Z. P. 1977. Some effects of oral doses of oxytetracycline on growth, survival and diseases in *Penaeus aztecus. Aquaculture*, **11(4)** : 355-362.

Costa-Pierce, B. A. and Laws, E. A. 1985. Chemotactically active feed additives for prawn (*M. rosenbergii*). *Prog. Fish. Cult.*, **47(1)** : 59-60.

Cravedi, J. P., Blanc, D., Labail, P. Y. and Kaushik, S. J. 1993. Effect of Virginiamycin and related factors on growth, body composition and plasma growth hormone levels of rainbow trout. In. Kaushik, S. J. and Luquet, P. (eds.) *Fish Nutrition in Practice* : 433-436.

Cruz-Rique, L. E., Guillaume, J. and Van Wormhoudt, A. 1987. Squid protein effect on growth of four penaeid shrimps. *J. World. Aquaculture. Soc.*, **18** : 282-309.

*Cruz- Suarez, L. E. and Guillaume, J. C. 1983. Facteur de croissanee inconnu de la farina de calmar pour la crevette japonerise : localisation da ce facteur conscil International pour I' Exploitation de la Mer CM. 1983 / F: 14.

Cruz-Suarez, L. E., Guillaume, J. C. and Van Wormhoudt, A. 1984. Effect of various levels of squid protein on growth and some biochemical

parameters of *Penaeus japonicus* juveniles. *Nippon Suisan Gakkaishi*, **53(11)** : 2083-2088.

Cruz-Suarez, L. E., Marie, D. R., Vega, J. A. M., Ebeling, P. W. 1993. Evaluation of two shrimp by-product meal as protein sources in the diet for *Penaeus vannamei*. *Aquaculture*, **115** : 53-62.

Cruz-Suarez, L. E., Rique, D. and AQUACOP. 1992. Effect of squid meal on growth of *Penaeus monodon* juveniles reared in ponds, pens and tanks. *Aquaculture*, **106(3-4)** : 293-299.

D' Abramo, L.R. and Sheen, S. 1993. Polyunsaturated fatty acid nutrition in juvenile freshwater prawn *Macrobrachium rosenbergii*. *Aquaculture*, **115** : 63-86.

Dabrowski, K. and Glogowski, J. 1977. A study of the application of proteolytic enzymes to fish food. *Aquaculture*, **12** : 349-360.

Dall, W. 1968. Food and feeding habits of some Australian Penaeid shrimp. *FAO Fisheries Reports*, **57(2)** : 251-258.

Danulat, E. 1986. Role of bacteria with regard to chitin degradation in the digestive tract of the cod *Gadus morhua*. *Mar. Biol.*, **90** : 335-343.

Danulat, E. and Kausch, H. 1984. Chitinase activity in the digestive tract of the cod, *Gadus morhua* (L.). *J. Fish. Biol.*, **24** : 125-133.

Das, N.K., Ghosh, A.N and Chakraborti, N.M. 1982. Food of some penaeid prawns relative to their culture in brackishwater ponds of sunderbans. Proc. *Symp. Coastal. Aquacul. Mar. Biol. Assn. India.*, **1** : 202-205.

Degani, G.,Ben-Zvi, Y. and Levanon, D. 1989. The effect of different protein levels and temperature on feed utilization, growth and body composition of *Clarius gariepinus* (Burchell- 1822). *Aquaculture*, **76** : 293-302.

Dempsey, A. C. and Kitting, C. L. 1987. Characteristics of bacteria isolated from penaeid shrimp. *Crustaceana*, **52(1)** : 90-94.

Deshimaru, O. and Kuroki, K. 1974. Studies on purified diet for prawn II. Optimum contents of cholesterol and glucosamine in the diet. *Bull. Jap. Soc. Sci. Fish.*, **40(4)** : 421-424.

Deshimaru, O. and Shigueno, K. 1972. Introduction to the artificial diet for prawn *Penaeus japonicus*. *Aquaculture*, **1(2)** : 115-133.

Deshimaru, O. and Yone, Y. 1978. Effect of dietary supplements on feeding behaviour of prawn. *Bull. Jap. Soc. Sci. Fish.*, **44(4)** : 903-905.

Dileo Craetano Pressman. 1987. The effect of monensin on growth of *P. vannamei*. *J. World. Aquacult. Soc.*, **24(4)** : 26A.(Abstract).

*Dobos, R. J., Savage, D. C. and Schaedler, R. 1967. The indigenous flora of gastrointestinal tract. *Dis. Colon Rectum.*, **10** : 23-34.

*Dokken, Q. R. and Lawrence, A. R. 1985. Growth response of *Penaeus vannamei* to feeding frequency and dietary ingredients, soybean meal, white meal mixture, squid meal and squid pusee. Presented at the Meeting of the World Mariculture Society, January 13-17, 1985. Orlando, Florida, U. S. A.

Donaldson, E. M., Fagerlund, V. H. M., Higgs, D. A. and Mc Bride, J. R. 1979. Hormonal enhancement of growth. In. Hoar, W.S., Randall, D. J. and Brett, J. R. (eds) *Fish Physiology*. Vol 8. Academic press, London, P. 456-578.

Dy Penaflorida, V. 1995. Growth and survival of juvenile tiger shrimp fed diet where fish meal is partially replaced with papaya (*Carica papaya* L) or Camote (*Ipomea batatas* Lam) leaf meal. *Isr. J. Aquaculture*, **47(1)** : 25-33.

E.E.C. 1985. Official journal of European communities. (Legislation) No. L255 / 12.9.85, Vol. **28**, P. 1-32.

Elyakowa, L. A. 1972. Distribution of cellulases and chitinases in marine invertebrates. *Comp. Biochem. Physiol.*, **43** : 67-70.

Factor, J. R. and Dexter, B. L. 1993. Suspension feeding in larval crabs (*Carcinus maenas*). *J. Mar. Biol. Assoc.* ,**73** : 207-211.

Fair, P. H., Fortner, A. R., Millikin, M. R. and Sick, L. V. 1980. Effects of dietary fibre on growth, assimilation and cellulase activity of the prawn (*Macrobrachium rosenbergii*). *J. World. Maricult. Soc.*, **11**: 369-381.

Fange, R., Lind, L. J. and Slettengren, K. 1979. Chitinolytic enzymes in the digestive system of marine fishes. *Marine Biol.*, **53 :** 317-321.

Febvay, G., Decharmc, M. and Kermarree, A. 1984. Digestion of chitin by the labial glands of *Acromyrmex octospinosus* Reich (Hymenoptera : Formicidae). *Can. J. Zool.*, **62** : 229-234.

Fenton, D., Davis, B., Rotgers, C. and Eveleigh, D. E. 1978. Enzymatic hydrolysis of chitosan. In Muzzarelli, R. A. and Pariser, E. R. (eds.) *Proc. First International conf. chitin/chitosan*. Boston, April 11-13, 1977 : 554-561.

Forster, J. R. M. and Gabbot, P. A. 1971. The assimilation of nutrients from compounded diets by the prawns *Palaemon serratus* and *Pandalus platyceros*. *J. Mar. Biol. Assoc. U. K.*, **51** : 943-961.

*Foster, J. R. M. and Beard, T. W. 1973. Growth experiments with the prawn *Palaemon serratus* fed with fresh and compounded foods. *Fish. Invest. Ser.*, **11(7)** : 16-20.

Foster, J. R. M. and Beard, T. W. 1974. Experiments to assess the suitability of nine species of prawns for intensive cultivation. *Aquaculture* ,**3** : 355-368.

Fox, C. J. 1993. The effect of dietary chitin on the growth survival and chitinase levels in the digestive gland of juvenile *Penaeus monodon* (Fab.). *Aquaculture*, **109** : 39-49.

Frakenburg, D. and Smith, D. E. 1967. Coprophagy in marine animals. *Limnol. Oceanography.*, **12** : 443-450.

Gahuam, A. L. and Lovell, R. T. 1991. Effect of feeding 17 α- methyl testosterone, 11-ketotestosterone, 17 β- estradiol and 3,5,3'-triiodo thyronine to channel cat fish *Ictalurus punctatus*. *Aquaculture,* **92(4)** : 377-388.

*Garriques, P. and Arevalo, G. 1995. An evaluation of the production and use of a live bacterial isolate to manipulate the microbial flora in the commercial production of *P. vannamei* post larvae in Ecuador. World Aquaculture Meeting, 1-4 February 1995, San Diego, USA (Abstract).

Gatesoupe, F. J., Arakawa, T. and Watanabe, T. 1989. The effect of bacterial additives on the production rate and dietary value of rotifers as food for Japanese flounder, *Paralichthys olivaceus*. *Aquaculture,* **83** : 39-44.

George, M.J. 1972 b. On the food and feeding habits of *Metapenaeus monoceros* (Fabricius). *Fishing News Books Ltd.*, London., P. 178 .

Gogoi, D. and Keshavanath, P. 1988. Digestibility and growth promoting potential of 17-α methy testosterone incorporated diets in the Mahseer, *Tor khudree*. In. Joseph, M. M. (ed.) *The Third Indian Fisheries Forum Proceedings* : 99-102.

Goodrich, T. D. and Morita, R. Y. 1977 a. Incidence and estimation of chitinase activity associated with marine fish and other estuarine samples. *Mar. Biol.*, **41** : 349-353.

Goodrich, T. D. and Morita, R. Y. 1977 b. Bacterial chitinase in the stomachs of marine fishes from Yaquina Bay, Oregon, U.S.A. *Mar. Biol.*, **41** : 355-360.

Gopalakrishnan, V. 1952. Food and feeding habits of *P. indicus* Milne Edwards. *J. Madras Univ.*, **22 B (1)** : 69-75.

Gyorgy, P., Kuhn, R. and Zilliken, F. 1955. U.S. patent, 2, 710, 806. *Chem. Abstr.*, **49**: 18550.

Hall,H. H. 1962. Microbiology in Animal nutrition. In. Umbreit, W. W (ed) Advances in Applied Microbiology, vol. **4**, P. 77-99.

Hanson, J. A. and Goodwin, H. L. 1977. Shrimp and prawn farming in the western hemisphere. Dowden, Hutchinson and Ross, Stroudsburg, P. A., P. 439 .

Harpaz, S., Kahazan, D. and Galun, R. 1987. Variability of feeding behaviour of the Malaysian prawn *Macrobrachium rosenbergii* (De Mann) during moult cycle (Decapoda, Crustacea). *Crustaceana*, **52** : 53-60.

Hartati, R. and Briggs, M. R. P. 1993. Effect of feeding attractants on the behaviour and performance of juvenile *Penaeus monodon* Fabricius. *Aquacult. Fish. Manage.*, **24** : 613-623.

Hasting, W.H. 1976. Fish nutrition and fish feed manufacture. FAO Tech. Conf. on Aquaculture, Japan. FIR : AQ/conf/76/R., **23** , P. 13.

Hatha, A. A. M. and Lakshmana Perumalsamy, P. 1995. Antibiotic resistance of Salmonella strains isolated from fish and crustaceans. *Lett. Appl. Microbiol.*, **21(1)** : 47-49.

*Hawkridge, J. 1978. Monensin- dose response relationship under European conditions. *Proc. Eur. Cong. for Improved Beef Productivity*. Paris, 28-29 Sept , 1978. Elanco Products Ltd., Windlesham, U. K.

Heinen, J. M. 1980. Chemoreception in decapod crustacea and chemical feeding stimulants as potential feed additives. *Proc. World. Maricult. Soc.*, **11** : 319-334.

Hepher, B. 1988. Nutrition of Pond Fishes. Cambridge University Press, Cambridge, P. 388.

Heyward, A. J. 1991. Growth hormones for fish larvae : can bioactive peptides be delivered to good effect. In. Lavens, P., Sorgeloos, P., Jaspers, E. and Oliver, F. (eds.) *Larvi-91.*, **15** : 14-15.

Higgs, D. A., Fagerlund, U. H. M., Eales, J. G. and Mc Bride, J. R. 1982. Application of thyroid and steroid hormones as anabolic agents in fish culture. *Comp. Biochem. physiol.*, **73(B)** : 143-176.

Hood, M. A. and Mayers, S. P. 1973. Microbial aspects of penaeid shrimp digestion. *Proc. Gulf. Carib. Fish. Inst. 26th Ann. Sess.* Oct. 1973, New Orleans. Uni. of Miami : 81-91.

Hood, M. A. and Mayers, S. P. 1974. Microbial aspects of penaeid shrimp digestion. *Gulf Carib. Fish. Digt.*, **26** : 81-91.

Hoverton, R. D., Okimoto, D. K. and Grau, E. G. 1988. Changes in the growth rate of *Oreochromis mossambicus* following treatment with the hormones, triiodothyrinine and testosterone. In. Pullin, R. S. V., Bhukaswan, T., Tonguthai, K., Maclean, J. L (eds.). *The Second Internat. Symp. on Tilapia in Aquaculture.*, Bangkok, Thailand., **15**, P. 598.

Howerton, R. D., Okimoto, D. K. and Grau, E. G. 1992. The effect of orally administered 17 α-metyl testosterone and 3,3' triiodo-L-thyronine on growth of sea water adapted tilapia, *Oreochromis mossambicus* (Peters). *Aquacult. Fish. Manage.*, **23(1)** : 123-128.

Intriago, P. and Jones, D. A. 1993. Bacteria as food for Artemia. *Aquaculture,* **113** : 115-127.

Jiamin, X., Aijie, C., Weifeng, L. and Dao zheng, L. 1989. Effect of several growth promoters on the growth of *Penaeus orientalis* Kishinouye. *Mar.Sci.*, **1** : 51-58.

James, T., Sherif, M., Nair, C. M., and Thampi, D. M. 1990. Evaluation of Spirulina fusiformes as a protein source in the diets of post larvae of *Macrobrachium rosenbergii. Proc. Nat. Symp. Freshwater prawns*, Faculty of Fisheries, Kerala Agri. Uni., Kochi, 1990 : 234-237.

Jancarik, A. 1964. Die verdauung der hauptnahrstoffe beim karpfen. zt. Fisch. deren. *Hilfswiss*, **12** : 601-684.

*Jasmine, G. I., Pillai, S. P. and Athithan, S. 1993. Effect of ~~feed stimulant~~s on ~~the~~ biochemical composition and growth of Indian white prawn *Penaeus indicus.* Presented at : World Aquaculture '93 Int. Conf. From Discovery to Commercialisation. Carrillo, M., Dahle, L., Morales, J., Sorgeloos, P., Svennevig, N. and Wyban, J. (eds.) *Spl. Publ. Eur. Aquacult. Soc.* No. 19. (Summary).

Jauncey, K. 1982. The effects of varying dietary protein levels on the growth, food conversion, protein utilization and body composition of juvenile tilapias (*Sarotherodon mossambicus*) *Aquaculture,* **27** : 43-54.

Jayaprakas, V. and Bindu, S. S. 1996. Effect of hormones on growth and food utilization of the Indian major carp, *Cirrhinus mrigala. Fishery Technology*, **33(1)** : 21-27.

Jayaprakas, V. and Sambhu, C. 1996. Effect of dietary hormones on food utilization, growth and body composition of the pearl spot, *Etroplus suratensis* (Bloch) in a brackish water pond. *Ind. J. Mar. Sci.*, **24** : 32-36.

Jeuniaux, C. 1966. Chitinases. In. Neufeld, E. F and Ginsher, V (eds.). *Methods in Enzymology*, **8**, Academic Press, Cambridge, P. 644-650.

Jeuniaux, C. and Cornelius, C. 1978. Distribution and activity of chitinolytic enzymes in the digestive tract of birds and mammals. In. Muzarelli, K. A. and Pariser, E. R. (eds.) *Proc. First Internat. Conf. chitin/chitosan* : 542-549.

*Josekutty, P.A. and Susheela Jose. 1992. Growth response of *Penaeus monodon* to pelleted feeds of different protein sources. Presented at: National seminar on prawn feeds; 25-26 Feb. 1992.

Josemon, P. K. 1990. Growth increments and conversion efficiency of juvenile *Penaeus indicus* (H. Milne Edwards) fed on artificial diets supplemented with testosterone propionate. M. Phil. Dissertation; Annamalai University, P. 190.

Jukes, T. H. 1973 The present status and background of antibiotics in the feeding of domestic animals. *Ann. N. Y. Acad. Sci.*, **182** : 362-378.

Kanazava, A. 1990. Recent advances in penaeid nutrition in Japan. In. Allan, G. C. and Dall, W. (eds.) *Proc. Aquaculture Nutrition Work Shop*. NSW Fisheries, Australia 1991 : 64-71.

Kanazava, A. 1995. Recent developments in shrimp nutrition and feed industry. Paper presented at INDAQUA, January 1995, Madras, India.

Kanazava, A., Shimaya, M., Kawasaki, M. and Kashiwada,K. 1970. Nutritional Requirements of Prawn-I. Feeding on Artificial diet. *Bull. Jap. Soc. Sci. Fish.*, **36(9)** : 949-954.

Kanazava, A., Tanaka, M. and Kashiwada, K. 1972. Nutritional requirement of prawn IV : The dietary effect of ecdysones. *Bull. Jap. Soc. Sci. Fish.*, **38(9)** : 1067-1071.

*Kent, A.W. and White house, H.W. 1955. *Biochemistry of amino sugars*. Academic press Inc., New York.

Keshavananth, P. and Matty, A. J. 1994. Growth promoting potential of human chorionic gonadotropin (HCG) in common carp *Cyprinus carpio* (L). *Aquacul. Fish. Manage.*, **25(9)** : 881-889.

Keshavanath, P., Shyama, S., Nandeesha, M. C. and Verghese, T. J. 1991. Influence of Virginiamycin on growth and body composition of Rohu (*Labeo rohita*) and Common carp (*Cyprinus carpio*). In. S. S. De Silva (ed.)

Fish nutrition research in Asia. Proc. of the Fourth Asian Fish Nutrition Workshop. Asian Fisheries Society, Sp. Publ. 5, Asian Fisheries Society, Manila, Philippines : 193-200.

Kitabayashi, K., Kurata, H., Shudo, K.,Nakamura, K. and Ishikawa, S. 1971. Studies on formula feed for kuruma prawn I. On the relationship among glucosamine, phosphorus and calcium. *Bull. Tokai Reg. Fish. Res. Lab.*, **65** : 91-107.

Koga, D., Mizuki, K., Ide, A., Kono, M., Matsui, T. and Shimizu, C. 1990. Kinetics of a chitinase from a prawn, *Penaeus japonicus. Agric. Biol. Chem.*, **54** : 2505-2512.

Kono, M., Matsui, T. and Suimizu, C. 1987. Effect of chitin, chitosan and cellulose as diet supplements on the growth of cultured fish *Nippon suisan Gakkaishi.*, **53(1)** : 125-129.

Kono, M., Matsui, T., Shimizu, C. and Koga, D. 1990. Purification and some properties of chitinase from the liver of a prawn. *Penaeus Japonicus. Agric. Biol. Chem.*, 54 : 2145-2147.

Krueskemper, H. L. 1968. *Anabolic steroids.* Academic Press Inc., New York, NY, P. 365 .

Lam, T. J. and Sharma, R. 1985. Effect of thyroxine and salinity on larval survival, growth and development in carp *Cyprinus carpio. Aquaculture,* **42** : 225-235.

Lam, T. J., Juario, J. V. and Banno, J. 1985. Effect of thyroxine on growth and development of post yolk sac larvae of milk fish *Chanos chanos. Aquaculture,* **46** : 179-184.

*Lee, B. M. 1988. Study on the digestive physiology of *Penaeus monodon* in different developmental stages. Masters thesis. National Sun Yat-Sen University, Kaohgiung, Taiwan (Abstract).

Lee, P. G. and Lawrence, A. C. 1982. A quantitative analysis of digestive enzymes in penaeid shrimp, influences of diet, age and species. *Physiologist,* **25** : 241-246.

Lee, P. G., Smith, L. L and Lawrence, A. L. 1984. Digestive proteases of *Penaeus vannamei* Boone, relationship between enzyme activity, size and diet. *Aquaculture,* **42** : 225-239.

Liao, I. C. 1977. A culture study on grass prawn *Penaeus monodon* in ~~Taiwa~~n the patterns, the problems and prospects. *J. Fish. Soc. Taiwan.*, **5(2)** : 11-29.

Liao, I. C. and Huang, H. J. 1975. Studies on the respiration of economic prawns in Taiwan I. Oxygen consumption and lethal dissolved oxygen of egg up to young prawns of *Penaeus monodon* Fabicius. *J. Fish. Soc Taiwan.* , **4(1)** : 33-50.

Lindsay, G. J. H., Walton, M. J., Adron, J. W., Fletcher, T. C., Cho, C. Y. and Cowey, C. B. 1984. The growth of Rainbow trout (*Salmo gairdneri*) given diets containing chitin and its relationship to chitinolytic enzymes and chitin digestibility. *Aquaculture,* **37** : 315-334.

Lovell, R. T., Lafleur, J. R. and Hoskins, F. H. 1968. Nutritional value of freshwater cray fish waste meal. *J. Agric. Food Chem.*, **16** : 204-208.

Luckey, T.D. 1959. *Antibiotics-Their chemistryand Non-Medical uses.* Van Nostrid, Princeton, New Jersey , P. 174-321.

Lynn, K. R. 1990. Chitinase and chitobiase from the American lobster (*Homarus americanus*). *Comp. Biochem. Physiol.* **96 B** : 761-766.

Madhavan, P. and Nair, K.G.R. 1974. Utilization of prawn waste- isolation of chitin and its conversion to chitosan. *Fishery Technology,* **11(1)** : 50-52.

Maeda, M. 1988. Microorganisms and protozoa as food in mariculture. *Prog. Oceanogra.*, **21** : 201-206.

Manik, R., Adisukresno, S. and Tiensongrusmee, B. 1979. The effect of high salinity on growth and survival of the giant tiger shrimp under cultivation in the earth pond. *Bull. Brackishwater Aquacult. Dev. Cent.*, **5(1&2)** : 351-361.

Marte, C.L. 1980. The food and feeding habits of *Penaeus monodon.* Fabricius collected from Makato river, Aklan, Philippines (Decapoda-Natantia). *Crustaceana,* **38 (3)** : 226-236.

Mathew, P. T., Nair, K. G. R. and Madhavan, P. 1987. Formulation of artificial diets for common carp (*Cyprinus carpio*). *Proc. Symp. Harvest and Post Harvest Technology of Fishes. Soc. Fish Tech.* : 628-630.

Mathew, P. T., Nair, K. G. R., Madhavan, P. and Prabhu, P. V. 1989. Effect of chitin and prawn shell on the growth of Albino rat. *Fishery Technology,* **26(1)** : 36-38.

Matty, A. J. and Lone, K. P. 1985. Hormonal control of protein deposition. In. Cowey, C. B., Mackie, A. M. and Bell. J. G. (eds.) *Nutrition and Feeding in Fish.* Academic Press, London, P. 147-167.

Maugle, P.D., Deshimaru, O., Katayama, T. and Simpson, K. L. 1983 a. The use of amylase supplements in shrimp diets. *J. World. Maricul. Soc.*, **14** : 25-37.

Maugle, P. D., Deshimaru, O., Katayama, T., Nagatani, T. and Simpson, K. L. 1983b. Effect of microencapsulated amylase and bovine trypsin dietary supplements on growth and metabolism of shrimp. *Bull. Jap. Soc. Sci. Fish.*, **49** : 1421-1427.

Mc Bride, J. R., Higgs, D. A., Fagerlund, U. H. M. and Bulkley, J. T. 1982. Thyroid and steroid hormone : Potential for control of growth and smoltification of salmonids. *Aquaculture,* **28** : 201-209.

Mc Donald, P. R., Edwards, R. A. and Greenhalgh, J. F. D. 1973. *Animal Nutrition.* Long man, London, P. 357-358.

Micha, J. C., Dandrifose, G. and Jeuniaux, C. 1973. Distribution et localisation tissulaire de la synthese des chitinase chez les vertebres inferieurs. *Arch. Internat Physiol. Bioch.*, **81** : 439-451.

*Mochizuki, H. 1979. *The present prawn culture in the Philippines.* Aquacult. Dept.,SEAFDEC, Tigbauam, Iloilo City.

Mohamed, K. H., Muthu, M. S., Pillai, N. N., Ahamad Ali, S. and Pandian, S. K. 1983. A simplified hatchery technique for mass production of penaeid prawn seed using formula feed. *J. Ind. Fish.*, **30(2)** : 320-332.

Mohamed, K. S. 1995. Probiotics- An emerging concept in agriculture nutrition and disease control. *Seafood Exp. J.* , **26(1)** : 5-9.

Mohamed, K. S. 1996. Heterotrophic marine bacteria as supplementary feed for larval *Penaeus monodon. NAGA* , **19(1)** : 23-26.

Moore, P. R., Evenson, A., Luckey, T. D., Mc Coy, E., Elvejim, C. A. and Hart, C. B. 1946. Use of sulfasuxidine, streptathriein and streptomycin in nutritional studies with chick. *J. Biochem.*, **165** : 437-447.

Nair, K.G. R., Mathew, P. T., Madhavan, P. and Prabhu, P.V. 1993. Effect of feeding chitin on poultry. *Fish. Technol.*, **30(1)** : 24-27.

*Nair. K..G.R. 1981. Role of fishery waste in animal feeds. *Proc. Summer Institute on Nonnutritional diversified farm products and byproducts.* April 27 to May 26, 1981. CIFT, Cochin .

Nakajima, K. 1991. Dimethyl- β- propiothein, a potent moult stimulant for stripped prawn. *Bull. Jap. Soc. Sci. Fish* , **57(9)** : 1717-1722.

Nanjundappa, T. and Verghese, T. J. 1988. Effect of diethyl stilbistrol on growth and food conversion of *Labeo rohita* (Ham). *Proc. the First Indian Fish. Forum.* Asian Fisheries Society, Indian branch, Mangalore : 79-80.

Navas, K. A. 1988. Effect of low salinities on the growth and survival of *Penaeus monodon* Fabricius.M. F. Sc. Thesis, Kerala Agri. Uni., P. 82.

New, M.B. 1976. A review of dietary studies with shrimps and prawns. *Aquaculture,* **9**: 101-144.

Nirmala, A. R. C. and Pandian, T. J. 1983. Effect of steroid injection on food utilization in *Channa striatus. Proc. Ind. Acad. Sci. (Ani. Sci.),* **92(3)** : 221-229.

Noor-Hamid, S., Fortes, R. D. and Parado-Estepa, F. D. 1992. Effect of pH and ammonia on survival and growth of the early stages of *Penaeus monodon* Fabricius. *Aquaculture,* **125** : 67-72

Oke, O. L., Talabi, S. O. and Umoch, I. B. 1978. The possible use of chitin and chitosan as animal feed In. Muzarelli, R. A. and Pariser, E. R (eds.). *Proc. First Internat. Conf. chitin/chitosan* : 327-333.

Okutani, K. 1966. Studies on chitinolytic systems in the digestive tract of *Lateolabrax japonicus. Bull. Misaki. Mar. Biol. Inst.*, **10** : 1-47.

Okutani, K., Hayasaki, Y. and Kimata, M. 1964. Studies on chitin decomposing bacteria in Japanese sea bream. *Lateolabrax japonicus. Bull. Jap. Soc. Sci. Fish.*, **30(11)** : 926-929.

Parado-Estepa, F. D., Quinitio, E.T. and Borlongan, E. L. 1990. *Prawn hatchery operation.* SEAFDEC Extension Manual, **19** , P. 44 .

*Pascual, F. P. 1980. Attractants in purified diets. *Quart. Res. Rep.* **IV(2)**, *SEAFDEC*, Tig baun, Iloilo, Philippines.

*Pascual, F. P. and Destajo, W. 1978. Growth and survival of *Penaeus monodon* post larvae fed on shrimp head meal as primary animal source of protein. *Q. Res. Rep. Aquacult. Dept., SEAFDEC* ,**2** , P. 26 .

Paul raj, R. 1993. *Aquacultural feed.* Hand book on Aquafarming. The Marine Products Exports Development Authority, Kochi, India, P. 93.

Pearson, D. 1976. *The chemical analysis of food.* Churchill, London, P. 575.

Pelissero, C. and Sumpter, J. P. 1992. Steroids and steroid like substances in fish diets. *Aquaculture,* **107** : 283-301.

Peres, G., Boge, G., Colin, D. and Rigal, A. 1973. Effects de la temperature sur les processus digestifs des poissons activities enzymatiques et absorption intestinale. *Rev. Trav. Inst. Peches. Marit.*, **37** : 223-232.

Perry, T. W., Beeson, W. M. and Mohler, M. T. 1976. Effect of monensin on beef cattle performance. *J. Ani. Sci.*, **42** : 761-765.

Pillai, S. M., Verghese, P. V., Ravichandran, P. and Roy, A. K. 1987. Effect of thyroxine on growth and moulting of *Penaeus monodon* Fab. *Ind. J. Ani. Sci.*, **57(3)** : 241-245.

*Raghunathan, C. and Ajmal khan, S. 1992. Growth increment and conversion efficiency of post larvae of *Penaeus indicus* fed on artificial diets supplemented with ethyloestrenol. *Nat. Sem. On Prawn feeds and Nat. Workshop on the impact of coastal Aquaculture on the environment.* The Fisheries College and Research Institute. *Tamil Nadu. Vet. and Ani. Sci. Uni.* Tuticorin and A. F. S. Indian branch.

Raman, K., Sultana Munavar and Abdul Kadir, P. M. 1982. Evaluation of supplementary feed for *Penaeus indicus*. *Proc. Symp. Coastal Aquaculture , Mar. Biol. Assn. India.*, **1** : 337-343.

Rao, D. S., Pillai, P. P., Mathew, K. J., Rengarajan, K., Vincent, D. and Khambadkar, L. K. 1983. Alfalfa promotes growth in prawns. *Mar. Fish. Inform. Ser., T & E Ser.*, **53** : 17-19.

Ravichandran, P., Verghese, P. V., Karmaker, H. C. and Rajyalakshmi, T. 1982. Effect of stocking density and size of *Penaeus monodon* Fabricius post larvae in nursery rearing experiments. *Proc. Symp. Coastal Aquacul., Mar. Biol. Assn. India.*, **1**. 412-413.

Reinitz, G. L., Orme, L. E., Lemm, C. A. and Hitzel, F. N. 1978. Influence of varying lipid concentrations in diets for rainbow trout (*Salmo gairdneri*). *Trans. Ani. Fish. Soc.*, **107** : 751-754.

Rijikers, G. T., Teunissen, A. G., Osterom, R. and Van Muiswinkel, W. B. 1980. The immune system of cyprinid fish. The immune suppressive effect of the antibiotic in carp (*Cyprinus carpio*). *Aquaculture*, **19** : 177-189.

Rossenberry, B. 1995. *World shrimp farming 1995*. Annual Report, Shrimp News International , San diego, P. 68 .

*Rupley, J. A. 1964. The hydrolysis of chitin by concentrated hydrochloric acid and the preparation of low molecular weight substrate for lysozymes. *Biochim. Biophys. Acta.*, **83** : 245-251.

*Salini, G. 1993. Impact of human chorionic gonadotropin and testosterone propionate on the growth, food utilization and body composition of *Labeo rohita* and *Catla catla*. M Sc. dissertation, Uni. of Kerala, Trivandrum.

Sambhu, C. and Jayaprakas, V. 1994 b. Effect of hormones on growth, food conversion and proximate composition of the white prawn, *Penaeus indicus* (Milne Edwards). *Ind. J. Mar. Sci.*, **23** : 232-235.

Santandren, I. A. and Diaz, M. F. 1994. Effect of 17 α- methyl testosterone on growth and nitrogen excretion (*Oncorhychus masou* Brevoort). *Aquaculture.*, **124(1-4)** : 321-333.

Santiago, C . R. 1991. Growth, survival and feed conversion of Nile tilapia fingerlings fed diets containing Bayo-n-ox, a commercial growth promoter. *Isr. J. Aquaculture. / Bamidegh* **43(2)** : 77-81.

*Sasai, K. 1981. A better experience in grass prawn culture. *Fish. Cult.*, **18(1)** : 63-67.

Satoh, H. and Nimura, Y. 1991. Growth promotion in Japanese eel by oral administration of an estrogen (diethyl stilbistrol). *Bull. Jap. Soc. Sci. Fish.*, **57(1)** : 21-27.

Sebastian, M. J., Thampi, D. M. and Rajendran, C. G. 1980. Preliminary experiments in tiger prawn culture and a report on seed prospecting of penaeid prawns in Kerala backwaters. *Proc. First. Nat. Symp. Shrimp. Farming.*, Bombay : 205-208.

Sedgewick, K. W. 1979. Influence of dietary protein and energy on growth, food consumption and food conversion efficiency in *Penaeus merguiensis* De Man. *Aquaculture,* **16** : 7-30.

Seki, H. and Taga, N. 1963. Microbiological studies on the decomposition of chitin in marine environments. 5. Chitinoclastic bacteria as symbionts. *J. Oceanogr. Soc. Japan.*, **19** : 38-41.

Sera, H. and Okutani, K. 1968. Studies on chitinolytic enzymes present in aquatic animals- VII. The chitinolytic enzyme present in a sea bream, *Acanthopagrus schlegeli*. *Bull. Jap. Soc. Sci. Fish.*, **34(10)** : 920-924.

Sheen, S. and D' Abramo, L. R. 1989. Response of juvenile freshwater prawn *Macrobrachium rosenbergii* to different levels of cod liver / corn oil mixture in a semipurified diet. *Aquaculture.*, **93** : 121-134.

Shiau, S. Y. and Chou, B. S. 1991. Effect of dietary protein and energy on growth performance of tiger shrimp *Penaeus monodon* reared in sea water. *Nippon Suisan Gakkaishi,* **57** : 2271-2276.

*Shiau, S. Y. and Chou, B. S. 1992. The supplementation value of taurine on the growth of *Penaeus monodon*. Aquaculture- '92. Growing towards the 21st century. Paper presented at: *The 23rd Ann. Conf. of World Aquacult. Soc.*, Orlando, Florida, May 21-25, 1992.

Shiau, S. Y. and Chou, B. S. 1994. Grass shrimp *P. monodon* as influenced by taurine supplementation. *Comp. Biochem. Physiol.*, **108 A(1)** : 137-142.

*Shiau, S. Y. and Peng, C . Y.1991. Utilization of different carbohydrates in grass prawn *Penaeus monodon* at different dietary protein level reared in seawater. Paper presented at: *The 22nd Ann. Conf. and Exposition, World. Aquacult. Soc.*, San Juan, Puerto Rico, June 16-20.

Shiau, S. Y., Knock, C. C. and Chou, C. C. 1991 a. Optimal dietary protein level of *Penaeus monodon* reared in sea water and brackishwater. *Nippon Suisan Gakkaishi,* **57** : 711-716.

Sri Prakash., Wishand, S. K. and Saxena, R. K. 1987. On breeding and rearing of fresh water prawn, *Macrobrachium chopraii* in closed water system. *Ind. J. Fish.*, **34** : 374-381.

Shyama, S. and Keshavanath, P. 1990. Growth response of Mahseer (*Tor khudree*) and silver carp (*Hypophthalmichthys molitrix*) to steroid incorporated diets. *J. Aquacult. Trop.*, **5** : 155-159.

Sick, L. V. and Andrews, J.W. 1973. The effect of selected dietary lipids, carbohydrates and proteins on the growth, survival and body composition of *Penaeus duorarum*. *Proc. World. Mari. Soc.*, **4**: 263-276.

Sick, L.V., Andrews, J.M. and White, D.B. 1972. Preliminary studies on selected environmental and nutritional requirements for the culture of penaeid shrimp. *Fish. Bull.*, **70** : 101-109.

Simpson, K. L., Katayama, T. and Chinchester, C. O. 1981. Carotenoids in fish feeds. In. Bauerfeind, J. C (ed.). *Carotenoids as colourants and vitamin A precursors*. Academic Press, New York: 463-538.

Smith, B. R., Miller, G. C. and Mead, R. W. 1987. Taurine tissue concentrations and salinity effect on taurine in the fresh water prawn *Macrobrachium rosenbergii* (De Mann). *Comp. Biochem. Physiol.*, **87A** : 907-909.

Snedecor, G.W. and Cochran, G. 1968. *Statistical Methods*. Oxford and IBH Publishing Co., New Delhi, P. 593.

Spindler-Barth, M., Van Wormhoudt, A. and Spindler, K. D. 1990. Chitinolytic enzymes in the integument and midgut gland of the shrimp *Palaemon serratus* during the moulting cycle. *Mar. Biol.*, **106** : 49-52.

Steffens, W. 1981. Protein utilization by rainbow trout (*Salmo gairdneri*) and carp(*Cyprinus carpio*). A brief review. *Aquaculture*, **23** : 337-345.

Steffens, W. and Albrecht, M. L.1975. Der Einfluss des zus tzes unterschiedlicher fette zum Troskeumisch futter anf wachustum and Futter verwertung von Rogen bogenforellen (*Salmo gairdneri*). *Arch. Tierernahr.*, **25** : 717-723.

*Steffens, W. and Albrecht, M. L. 1979. Einsatz von harten Felt im Trockenmischfutter fur regenbogen forellon (*Salmo gairdneri*) 1. Mitt. Zuwachs and Futtervawctung bci der setzling saunfzucht. *Arch. Tierernahr.*, **29** : 597-604.

*Stuck, K. C., Overstreet, R. M. and Lotz, J. M. 1992. Effect of antibiotics on the growth and survival of *P. vannamei* in a small scale experimental system. Aquaculture . 92- Growing towards 21st century. Paper presented at: *The 23rd. Ann. Conf. of the World. Aquacult. Soc.* Orlando, Florida, May 21-25, 1992.

Subramanyam, M. 1973. Experimental studies on growth in *Penaeus monodon* Fabricius. *Proc. Semi. on Maricult. and Mech. Fishing* : 70-73.

Sundararajan, D., Bose, V. S. and Venkatesan, V. 1979. Monoculture of tiger prawn *Penaeus monodon* Fabricius in a brackish water pond at Madras. *Aquaculture*, **16(1)** : 73-75.

Surya Narayana and Alexander, K.M. 1972. Biochemical investigations ~~on edi~~ble molluscs of Kerala. I. A study on the nutritional value of ~~some bivalv~~es. *Fish. Tech.*, **9 (1)** : 42-47.

Takei, M. and Ai, N. 1971. Studies on fish's favourite foods VI. Response of walking legs to substance in kuruma prawn. *Bull. Tokai. Reg. Fish. Res. Lab.*, **57** : 61-69.

Takeuchi, T., Watanabe, T. and Ogino, C. 1978 b. Optimum ratio of protein to lipid in diets of rainbow trout. *Bull. Jap. Soc. Sci. Fish.*, **44** : 683-688.

Toullec, J. Y., Moullac, G. V., Cuzon, G. and Van Wormhoudt. A. 1991. Immunosensitive human growth hormone like peptide in tropical ~~penaeid~~s and the effect of dietary hGH on *Penaeus vannamei* larval development. *Aquat. Living. Resour.*, **4** : 125-132.

Tracey, M.V. 1951. Cellulase and chitinase in earth worms. *Nature.*, **167** : 776-777.

Trevino-Carrillo, L., Cruz-Suarez, L.E., Rique, D., Higuera-Ciapara, I. and Noriega-Orozco, L. 1993. Influence of virginiamycin supplementation on food conversion, growth and survival of Penaeus vannamei juveniles in a semi-intensive grow out pond in Sinaloa, Mexico. In. Carillo, M., Dahle, L., Moraler, J., Sorgeloos, P., Svennevig, N. and Wyban, J. (eds.) *From Discovery to Commercialization,* European Aquaculture Society, **19** , P.177 .

Ung, E. H. and Junilla, M. 1988. Preliminary observations on the nutritional effects of a betaine / amino acid mixture : survival, growth and food conversion of juvenile *P. monodon* fed with FINNSTIM. Report on the work shop on shrimp and fin fish feed development. Malaysia. ASEAN / UNDP / FAO, MANILA, P.71-83.

*Utama Che and Musa Che. 1991. The growth promoting effect of Virginiamycin on the tiger prawn larvae. *Fish. Bull. Dep. Fish. Malaysia,* **71**, P. 7 .

Vaitheswaran, S. and Ali, S.A. 1986. Evaluation of certain substances as growth promoting agents for the prawn, *Penaeus indicus. Ind. J. Fish.*, **33**: 96-105.

Van Wormhoudt, A., Ceccaldi, H. J. and Martin, B. J. 1980. Adaptation of the level of hepatopancreatic digestive enzymes in *Palaemon serratus* (Crustacea-Decapoda) to the composition of experimental diets. *Aquaculture,* **21** : 63-78.

Vanbelle, M. E., Teller, E. and Focant, M. 1989. Probiotics in animal nutrition: a review. *Arch. Tierenaechr.*, **40** : 543-567.

Venkataramiah, A., Cook, D. W., Biesiot, P. and Lakshmi, J. 1978. Nutritional value of high marsh grass and shrimp shell waste for commercial brown shrimp (*Penaeus aztecus* IVES). *Proc. World. Maricul. Soc.*, **9** : 217-224.

Venkataramiah, A., Lakshmi, G. J. and Gunter, G. 1975. Effect of protein level and vegetable matter on growth and food conversion efficiency of brown shrimp. *Aquaculture* 6 : 115-125.

Verghese, T. J. and Gireesha, O. 1994. Use of antibiotics in fish feed. *Seafood Expo. J.*, **25(11)** : 41-44.

Viola, S. and Arieli, Y. 1987. Non hormonal growth promoters for tilapia and carp. I. Screening test in cages. *Bamidegh,* **39 (2)**: 31-38.

Visek, W. J. 1978. The mode of growth promotion by antibiotics. *J. Ani. Sci.*, **46(5)** : 1447-1469.

Wagner, E. D. 1954. The effect of antibiotics and arsanilic acid on the growth of rainbow trout fingerlings. *Prog. Fish. Cult.*, **16** : 36-38.

Wilson, R. P., Pue, W. E., Nemetz, T.G. and Mac Millein, J. R. 1988. Effect of recombinant bovine growth hormone administration on growth and body composition of channel cat fish. *Aquaculture,* **73** : 229-236.

Zoccarato, I., Gasen, L., Celvi, S. L. and Fortina, R. 1995. Effect of dietary ovoparcin on performances and carcass composition in rainbow trout, *Oncorhynchus mykiss* (Walbaum). *Aquaculture research.*, **26** : 361-366.

*Not referred in the original

EVALUATION OF GROWTH IN *PENAEUS MONODON* FABRICIUS BY INCORPORATION OF SELECTED NONHORMONAL GROWTH PROMOTERS IN THE DIET

BY
M.P.VINOD

ABSTRACT OF A THESIS

submitted in partial fulfilment of the requirement for the degree

MASTER OF FISHERIES SCIENCE

FACULTY OF FISHERIES

KERALA AGRICULTURAL UNIVERSITY
DEPARTMENT OF AQUACULTURE
COLLEGE OF FISHERIES
PANANGAD- KOCHI

1997

VIII. ABSTRACT

The Effect of three growth promoters viz., Chitin, chitosan and glucosamine each at three levels (0.25, 0.5 and 1g per 100g diet) were evaluated in *P.monodon* early juveniles for a period of 70 days. The three growth promoters at three level were tested with 3 replicates for each treatment. The growth promoters were incorporated into a soyflour clam meal based practical diet containing 40% protein and fed to the shrimps *ad libitum*.

The results showed that the overall growth was not affected by dietary inclusion of chitin though the growth rate at 0.25g chitin per 100g diet was comparatively better than that of the control diet. Chitin does not seem to have any effect on specific growth rate of the juveniles although incorporation at 0.25g/100g diet improved the food conversion of the animal significantly over the control. Percentage survival values were also not significant at any level of incorporation. It does not seem to improve protein efficiency ratio and productive protein value too.

Incorporation of chitosan at 0.25 and 0.5g/100g diet significantly improved the weight gain, specific growth rate, food conversion ratio, protein efficiency ratio and productive protein value. However inclusion of chitosan at 1g/100g diet did not have any effect on growth though it significantly improved food conversion ratio, protein fficiency ratio and productive protein value. None of these treatments had any effect on survival of the animal.

Incorporation of glucosamine into the diet at 0.25g/100g diet was found to be the most efficient amongst the different treatments as evidenced by various growth parameters. It was significantly different from all other treatments in terms of growth, productive protein value and protein efficiency ratio. However it was not significantly different from glucosamine incorporation at 0.5g/100g diet in terms of specific growth rate and food conversion ratio. Enhancement of glucosamine level to 1g/100g diet was found to give poor result compared to the control. The survival rate of shrimps were not significantly different among the treatments. Hence in the present study, glucosamine was found to be superior to chitin and chitosan as a growth promoter and the optimum level was identified as 0.25g/100g diet for *P. monodon* juveniles.